GRAZIELI DE FRANÇA DUELI

ECOLOGIA EVOLUTIVA DE PLANTAS E INSETOS ADAPTADOS A SOLOS METALÍFEROS

Tese apresentada à Universidade Federal de Viçosa, como parte das exigências do Programa de Pós-Graduação em Ecologia, para obtenção do título de *Doctor Scientiae.*

VIÇOSA
MINAS GERAIS - BRASIL
2019

Ficha catalográfica preparada pela Biblioteca Central da Universidade Federal de Viçosa - Câmpus Viçosa

T

D852e
2019

Dueli, Grazieli de França, 1978-
Ecologia evolutiva de plantas e insetos adaptados a solos metalíferos / Grazieli de França Dueli. – Viçosa, MG, 2019.
x, 77 f. : il. (algumas color.) ; 29 cm.

Texto em português e inglês.
Orientador: Sérvio Pontes Ribeiro.
Tese (doutorado) - Universidade Federal de Viçosa.
Inclui bibliografia.

1. Habitat (Ecologia). 2. Metais. 3. Candeia (Árvore).
4. Térmita. 5. Análise de sobrevivência (Biometria).
I. Universidade Federal de Viçosa. Departamento de Biologia Geral. Programa de Pós-Graduação em Ecologia. II. Título.

CDD 22. ed. 577

GRAZIELI DE FRANÇA DUELI

ECOLOGIA EVOLUTIVA DE PLANTAS E INSETOS ADAPTADOS A SOLOS METALÍFEROS

Tese apresentada à Universidade Federal de Viçosa, como parte das exigências do Programa de Pós-Graduação em Ecologia, para obtenção do título de *Doctor Scientiae*.

APROVADA: 11 de fevereiro de 2019.

Ricardo Ildefonso de Campos

Juraci Alves de Oliveira

Paulo Fellipe Cristaldo

Fernanda V. Costa

Fernanda Vieira da Costa

Servio Pontes Ribeiro
(Orientador)

GRAZIELI DE FRANÇA DUELI

ECOLOGIA EVOLUTIVA DE PLANTAS E INSETOS ADAPTADOS A SOLOS METALÍFEROS

Tese apresentada à Universidade Federal de Viçosa, como parte das exigências do Programa de Pós-Graduação em Ecologia, para obtenção do título de *Doctor Scientiae.*

APROVADA: 11 de fevereiro de 2019.

Ricardo Ildefonso de Campos	Juraci Alves de Oliveira
Paulo Fellipe Cristaldo	Fernanda Vieira da Costa

Servio Pontes Ribeiro
(Orientador)

À Elisa,
minha foguetinha!

AGRADECIMENTOS

Aos meus pais e às minhas irmãs, pelo amor e pelo apoio incondicional.

Ao Haroldo por todo amor, motivação e por contribuir efetivamente em todas as etapas desse trabalho.

Aos orientadores Sérvio e Og, pela confiança, direcionamento e oportunidade de crescimento acadêmido durante nossa convivência.

Aos professores da UFV (Ecologia e Entomologia) pela convivência e por terem contribuído imensamente para o meu aprendizado.

Aos meus cumpadres (Gizeli e Cid Clay) pelas idas a campo, pela busca por ninhos de cupins e pelo apoio incondicional.

À Fernanda Frida e ao Breno, pela imensa dedicação e compromisso com os experimentos.

À família Leeids: Glorinha, Gisa, Maria Fernanda, Victor, Gerson, Wilson, Isabela, Mariana; aos ex-Leeids (Cinthia, Talita, Tassia, Miriã, Letizia, Clara, Julia, Jean) e aos técnicos George e Jaci pela ajuda nos campos e no laboratório.

Aos professores Julio Fontenelle e Marcão e aos colegas Isabela (Leeids), Alessandra Marins, Cinthia pelas identificações dos coabitantes.

Ao “Seu” Mario, técnico do Horto Botânico – UFOP, pela atenção e presteza com que tratou a nossa equipe (e as candeias também!).

As instituições de fomento: Fapemig e CAPES.

Aos motoristas da UFOP que tanto nos ajudaram nos campos.

À Elisa, pela compreensão em passar as manhãs no Itacolomi e por alegrar minha vida!

Sem vocês esse trabalho não seria possível.

Meus sinceros agradecimentos, de coração!

Venha ver como é verde na floresta
Tantas árvores que a gente não consegue nem contar...”

SUMÁRIO

LISTA DE TABELAS

Table 1: Elements concentrations (mg/Kg) in Parque Estadual do Itacolomi (PEIT) and Padre Viegas (PV) soils, in Minas Gerais state, Brazil. Ratio PEIT/ PV means how many times the element occurs in the soil of the PEIT in relation to the soil of PV.

Table 2 - Percentage and volume of soil used in each combination to compond the 7 levels and metal concentration (mg/Kg) in the soils of each level at which the plants (*Eremanthus erythropappus*) were cultivated.

Table 3 - Candeia identification (Plant id); metal soil level (Soil level) that represents a metal gradient where level 1 is composed by 100% of Padre Viegas (PV) soil and level 7 by 100% Parque Estadual do Itacolomi (PEIT) soil; five blocks in which the plants were arranged in the fields (Blocks); growth rates at which gr_rate1 represents growth under the effect of metals but without the effect of herbivory and gr_rate2 represents the effect of metals and herbivory on plant growth; percentage of leaf area consumed (Leaf area cons (%) calculated on Image J software; number of attacked leaves (Attacked leaves) measured as the number os leaves presenting any sign of herbivory attack during 120 days of field experiment; metals concentration in plant tissue: aluminun (Al), coppper (Cu), iron (Fe), manganese (Mn) and zinc (Zn) in mg/Kg.

Table 4 - List of termitophiles found in the 16 nests of *Cornitermes cumulans* collected in PEIT (Itacolomi State Park) and Padre Viegas (PV) in Minas Gerais, Brazil.

Table 5 - List of inquilines species found in the 16 nests of *Corniterems cumulans* collected in Parque Estadual do Itacolomi (PEIT) and Padre Viegas (PV) soils in Minas Gerais, Brazil. Each number refers to sampled colony.

Table 6 - List of Formicidae species found in the 16 nests of *Corniterems cumulans* collected in Parque Estadual do Itacolomi (PEIT) and Padre Viegas (PV) soils in Minas Gerais, Brazil. Each number refers to sampled colony.

Table 7 - Higher (H), volume (V), cohabitants abundance (Abd) and richness (Riq) and metals concentration (Al, Cu, Fe, Mn and Zn) of each nest collect at Parque Estadual do Itacolomi (PEIT) and Padre Viegas (PV), Minas Gerais state, Brazil.

RESUMO

DUELI, Grazieli de França, D.Sc., Universidade Federal de Viçosa, fevereiro de 2019. **Ecologia evolutiva de plantas e insetos adaptados a solos metalíferos**. Orientador: Sérvio Pontes Ribeiro. Coorientador: Og Francisco Fonseca de Souza.

Solos metalíferos são importantes filtros ecológicos, restringindo o estabelecimento de espécies e beneficiando aquelas evolutivamente adaptados a eles. Os metais podem ser tóxicos e muitos organismos desenvolveram mecanismos para tolerar, excluir ou acumulá-los em seus tecidos. O limiar de toxicidade e deficiência dos elementos metálicos é diferente para cada espécie e mesmo organismos adaptados sofrem custos energéticos para metabolizar os metais do ambiente. Neste estudo, testamos cinco hipóteses sobre o efeito dos metais em plantas e insetos: (i) Candeia sofre um conflito entre bioacumular metais e crescer; (ii) a bioacumulação de metais pelas Candeias atua como uma defesa elementar contra herbívoros mastigadores; (iii) Candeias que crescem sob o estresse dos metais e sob ataque dos herbívoros podem ter seu crescimento comprometido; a concentração de metais nos ninhos afeta: (vi) a sobrevivência dos cupins construtores e (v) a invasão dos ninhos por coabitantes. Para testar essas hipótese, realizamos dois experimentos. No experimento 1, cultivamos 70 Candeias em solos com diferentes concentrações de metais e posteriormente submetemos as plantas a herbívoros em seu habitat natural. Medimos o crescimento das plantas, a porcentagem de área foliar consumida e o número de folhas atacadas e analisamos a concentração de metais nas folhas. Apesar da herbivoria ter sido extremamente baixa, as análises dos dados mostraram que nenhum metal teve efeito sobre a herbivoria. Porém, a herbivoria foi modulada pelos metais acumulados nas folhas e afetou o crescimento das Candeias. Alumínio em concentrações médias e altas afetou negativamente o crescimento das Candeias. O cobre em concentrações médias e altas modulou positivamente a interação, suprimindo os efeitos do alumínio em concentrações médias. Nossos resultados evidenciaram uma modificação na interação herbívoro-planta reduzindo o efeito do antagonismo, ao mostrar uma resposta positva das plantas (crescimento) ao ataque dos herbívoros, sob estresse ambiental. Experimento 2: Coletamos 16 ninhos de cupins em duas áreas com diferentes concentrações de metais. Identificamos os coabitantes, medimos a concentração de metais nos ninhos e avaliamos o tempo de sobrevivência dos cupins operários. A conentração de metais nos ninhos foi bem menor que as encontradas nos solos adjacentes aos ninhos. Curvas de sobrevivência de trabalhadores de cupins foram afetadas negativamente pela concentração de zinco e pela riqueza de espécies de coabitantes nos ninhos. O zinco e o manganês afetaram negativamente a riqueza e a abundância de coabitantes. Habitar solos metalíferos é bastante custoso para os cupins, que ao gastar energia selecionando partículas, tornam-se menos hábeis para defender o ninho. Isso implica em mais coabitantes em ninhos com menos metais, já que os coabitantes devem procurar ambientes menos tóxicos para habitarem e acabam encontrando nesses ninhos um ambiente mais fácil para entrar e se estabelecer. Ambientes metalíferos são difíceis de serem invadidos porque impõem um ajuste adaptativo custoso, porém, possível, garantindo possibilidades de benefícios adaptativos relacionados a esses ambientes.

ABSTRACT

DUELI, Grazieli de França, D.Sc., Universidade Federal de Viçosa, February, 2019. **Evolutionary ecology of plants and insects adapted to metalliferous soils.** Advisor: Sérvio Pontes Ribeiro. Co-advisor: Og Francisco Fonseca de Souza.

Metalliferous soils are important ecological filters, wich restric the establishment of species and benefit those evolutionarily adapted to them. Metals can be toxic and many organisms have developed mechanisms to tolerate, exclude or accumulate them in their tissues. The threshold of toxicity and deficiency of metal elements is different for each species and even adapted organisms suffer from energy costs to metabolize metals from the environment. In this study, we tested five hypotheses about the metals effect of on plants and insects: (i) Candeia suffers a conflict between bioaccumulating metals and growing; (ii) the mwetals bioaccumulation by Candeias acts as an elemental defense against chewing herbivores; (iii) Candeias that grow under the metal stress and under herbivores attack may have their growth compromised; the concentration of metals in the nests affects: (vi) the termite builders survival and (v) the nest invasion by cohabitants. To test these hypotheses, we performed two experiments. In experiment 1, we cultivated 70 Candeias in soils with different metal concentrations and later submitted the plants to herbivores in their natural habitat. We measured the plants growth, the percentage of leaf area consumed and the number of leaves attacked and analyzed the metal concentrations in the leaves. Although herbivory was extremely low, data analysis showed that no metal had an effect on herbivory. However, the herbivory was modulated by the metals accumulated in the leaves and affected the Candeia's growth. Aluminum in medium and high concentrations negatively affected the Candeia's growth. Copper at medium and high concentrations positively modulated the interaction, suppressing the effects of aluminum at medium concentrations. Our results evidenced a modification in the herbivore-plant interaction reducing the effect of the antagonism, when showing a positve plants response (growth) to the herbivorous attack, under environmental stress. Experiment 2: We collected 16 termite nests in two areas with different metal concentrations. We identified the cohabitants, measured the metal concentration in the nests and evaluated the survival time of termite workers. The metal concentration in the nests was much lower than those found in the adjacent soils to the nests. Termite workers survival curves workers were negatively affected by zinc concentration and cohabitants richness in nests. Zinc and manganese negatively affected the cohabitants richness and abundance. To inhabit metalliferous soils is very costly for the termites, that when spending energy selecting particles, they become less able to defend the nest. This implies more cohabitants in nests with less metals, since cohabitants should seek less toxic environments to inhabit and end up finding in these nests an easier environment to enter and establish. Metallic environments are difficult to invade because they impose a costly, but possible, adaptive adjustment, guaranteeing possibilities of adaptive benefits related to these environments.

Capítulo 1

Introdução Geral

Ambientes extremos e pressões seletivas

Plantas e insetos têm evoluído por milhões de anos para se adaptarem a um conjunto de condições estressantes imposto pelos diferentes ambientes. Assim, conseguiram se estabelecer em ambientes áridos, pobres em nutrientes, com temperaturas muito elevadas ou muito baixas e ricos em sais e em metais. Esses ambientes, extremos ou estressantes, oferecem condições persistentemente subótimas (Bohnert & Sheveleva 1988), atuando como barreiras que limitam a amplitude geográfica da distribuição das espécies, podendo manter a diversidade biológica baixa nesses locais (Kristijánsson & Hereggvidsoon 1995, Tilman 1999).

As pressões seletivas geradas pelos ambientes extremos são diversas e uma única região pode oferecer um conjunto delas, assim como uma pressão seletiva pode gerar mais de uma adaptação. Na Tundra e nos Alpes, por exemplo, a principal pressão seletiva envolve baixas temperaturas (Grime 1997), mas há também déficit de nutrientes (Bliss 1971, Grime 1997), dessecação por ventos secos, congelamento do solo (Grime 1997) e déficit hídrico (água líquida) durante o inverno (Bliss 1971). Nos Desertos quentes, a escassez de água, o alto índice de radiação e extremos de temperatura (Noy-Meir 1973, Cloudsley-Thompson 1975) atuam como os principais fatores estressantes. Nesses ambientes, os organismos ainda precisam lidar com a falta de sombreamento, presença de solos rochosos, ventos fortes e tempestades de areia (Cloudsley-Thompson 2001). Nos ambientes salinos, as principais pressões seletivas são os estresses osmótico e hídrico (Bohnert & Sheveleva 1988, Flowers *et al.* 2010, Bromham 2015), mas os organismos lidam também com aridez e poluição (Lutts & Lefèvre 2015). Há ainda os solos metalíferos, enriquecidos naturalmente com metais. Em geral, esses solos são também são rasos e pobres em nutrientes (Walker 1954, Yang *et al.* 1985, Brooks 1987, Brady *et al.* 2005, Vincent & Meguro 2008). Nesses ambientes, o principal estresse é o excesso de metais nos solos, exigindo dos organismos mecanismos de desintoxicação

(Baker 1981, Sibly & Calow 1989, Posthuma & Straalen 1993). Esses mecanismos podem ser externos ou internos. Os mecanism externos são: permeabilidade seletiva da membrana plasmática (Hall 2002, Krämer 2010), que reduz a absorção dos metais ou os bombeiam para fora da célula (Hall 2002). Esses transportadores de membrana podem ser codificados pela suprexpressão de genes em plantas hiperacumuladoras (Assunção *et al.* 2001, Pollard *et al*. 2002, Greenan 2009). Outro mecanismo externo se dá pela ação de micorrizas associadas às raízes que restringem a movimentação dos metais para as raízes hospedeiras (Jentschke & Godbold 2000). Já os mecanismos internos são: o acúmulo de metais nos vacúolos, onde não não há proteínas para serem degradadas e a quelação dos metais por ácidos orgânicos e proteínas (Krämer 2010, Hossain *et al.* 2012) como as metalotineínas (Janssen *et al.* 2009, Hodsonn 2012).

As condições estressantes aqui retratadas atuam como filtros ambientais (Keddy 1992) que podem afetar as interações entre os organismos (Bertness & Callaway 1994, Boyd & Martens 1998, Bertness *et al.* 1999, Callaway *et al.* 2002, Hodson 2012, He *et al.* 2013). O estresse causado pelo excesso de metais no solo, por exemplo, afeta as comunidades de diferentes formas: (i) reduz a diversidade funcional da comunidade microbiana do solo (Kandeler *et al.* 1996, Gadd 2010, Fernandes *et al.* 2011), afetando negativamente sua estrutura (Giller *et al.* 2009*)* e as rotas de ciclagem de nutrientes (Kandeler *et al.* 1996); (ii) reduz a diversidade de plantas (Hernández & Pastor 2008) e de insetos (Jones *et al.* 2010, Hodson 2012); (iii) afeta a estrutura das comunidades nas cadeias tróficas (Fleeger *et al.* 2003, Gall *et al.* 2015); (iv) afeta as interações presa-predador (McPherson *et al.* 2004) e (v) as interações hebívoro-planta (Koricheva *et al.* 1998). Koricheva *et al.* (1998) mostraram, por meio de uma meta-análise, que plantas sob diferentes tipos de estresse (estresse hídrico, poluição e sombreamento) tiveram suas interações com herbívoros variando em magnitude e direcionamento. Os autores encontraram que o desempenho de insetos em atacar plantas sob estresse pode variar dependendo da guilda dos insetos: desempenho positivo em sugadores e minadores e desempenho negativo em mastigadores e galhadores. Dessa forma, o estresse ambiental, assim como a resposta dos organismos ao ambiente podem ser determinante para a dinâmica estrutural de populações e comunidades e podem causar modificações em suas interações.

Ocorrência e disponibilidade de metais no solo

Com exceção dos radioisótopos, produzidos em reações nucleares, todos os metais estão presentes na Terra desde a sua formação (Walker & Hopkin 2006). Os solos e as rochas são as principais fontes naturais onde eles se encontram na Terra e sua ocorrência depende da composição da rocha matriz (King 1997, Bradl 2005, Oorts 2012), dos processos de formação do solo e das proporções e composições dos componentes da sua fase sólida (King 1997, Fadigas *et al.* 2002). É no horizonte B do solo que os metais ficam retidos devido à grande quantidade de argila e oxi-hidróxidos de ferro que os absorvem. É nesse horizonte, por exemplo, que são encontrados os nutrientes absorvidos pelas plantas (Bradl 2005) e os solos utilizados para construção de ninhos por cupins (Holt & Lepage 2002) e formigas (Tschinkel 2003).

As concentrações de metais nos solos são modificadas por processos pedogênicos e biogeoquímicos, por meio de entradas naturais como partículas de poeira derivadas do solo, rochas e cinzas vulcânicas e, principalmente, por atividades antrópicas (Alloway 2012). O solo também pode ser modificado por ação de seres vivos, como invertebrados, incluindo nematódios, micro e macroartrópodes, moluscos, anelídeos e cupins (Stork & Eggleton 1992, Lavelle *et al.* 2006). Cupins, no processo de construção dos seus ninhos, modificam as propriedades físico-químicas do solo ao transportarem partículas das camadas mais profundas, ricas em argila e cátions trocáveis para os horizontes mais superiores (De Bruyn & Conaches 1995, Holt & Lepage 2000). Eles aeram, alteram o pH, drenam e umidificam o solo. Também concentram mais nutrientes nas paredes dos ninhos, aumentando a heterogeneidade no ambiente (De Souza *et al.* 2012); selecionam tipos diferentes de solo (Jouquet *et al.* 2002) e partículas (Sarcinelli *et al.* 2013) para construção de seus ninhos, como C, N, P, Ca e Mg e excluem partículas tóxicas como alumínio (Al) (Sarcinelli *et al.* 2009, 2013). Além disso, os cupins alteram a capacidade de troca de cátions do solo (Fall *et al.* 2007), modificam a distribuição de nutrientes para as plantas, alterando a fertilidade do solo (Wood 1988) e promovendo o crescimento de plantas que vivem no entorno dos seus ninhos (Lee & Wood 1971, Holt & Lepage 2000). Todas essas alterações no solo, faz com que os ninhos promovam uma maior biodiversidade nos locais onde são construídos e alterem a disponibilidade dos macro e micronutrientes para os organismos, afetando também a disponibilidade e a distribuição dos íons metálicos.

Os metais ocorrem em diversas formas no solo: solúveis, trocáveis, precipitados com outros compostos, compondo a biomassa e complexados na matéria orgânica (King 1997). A absorção de metais pesados pelos organismos depende do elemento químico e da bioquímica de cada organismo. Para que um organismo absorva os metais, eles devem estar móveis para serem transportados e estarem disponíveis no ambiente (Bradl *et al* 2005). No solo, os principais processos químicos que afetam o comportamento e a biodisponibilidade de metais são aqueles causados pela adsorção de metais da fase líquida para a fase sólida, como: capacidade de troca de cátions (CTC), adsorção específica, complexação orgânica e co-precipitação (para descrição dos processos, ver: Evan 1989, Alloway 2012). As proporções relativas dos íons metálicos adsorvidos dependem do grau de acidez ou alcalinidade do solo (King 1997, Newman & Unger 2003, Walker *et al*. 2004), da sua composição mineralógica, como a presença de óxido de ferro (Fe) e manganês (Mn), do seu conteúdo de matéria orgânica (King 1997, Evans 1989, Basta *et al.* 2005) e das propriedades físico-químicas, tais como eletronegatividade e potencial iônico (Bradl *et al.* 2005). Um solo com acúmulo de matéria orgânica e pH elevado, por exeplo, resulta em alta adsorção de cobre (Cu), Fe, zinco (Zn) e Mn (Baker 1990).

Efeitos dos metais nos organismos e adatpações evolutivas

Quando absorvidos, os metais causam efeitos que são bem conhecidos e tem sido descritos em todos os níveis de organização biológica, de organismos a ecossistemas e em diferentes táxons. Em geral, eles prendem-se a componentes vitais da célula como proteínas estruturais, enzimas e ácidos nucleicos, interferindo em suas funções (Landis

& Yu 2003, Walker & Hopkin 2006). Eles podem inibir processos como fotossíntese, respiração, reduzir o vigor ou inibir o crescimento em plantas (Levitt 1980, Marschner 2002, Walker & Hopkin 2006). Em animais, os principais efeitos são os neurofisiológicos que levam a mudanças de comportamento, podendo afetar a reprodução (Hall 2002, Augustyanik *et al*. 2006, Walker & Hopkin 2006).

Alguns organismos, como plantas e invertebrados evoluíram estratégias para lidar com o excesso de metais no ambiente. As plantas podem tolerar/excluir, acumular e até hiperacumular metais em seus tecidos (Baker 1981, Ernest 2000, Pollard *et al.* 2002), usando-os como defesa elementar contra herbívoros (Martens & Boyd 1994, Mathews *et al.* 2009, Boyd & Martens 1998, Boyd 2012). Já os invertebrados, possuem mecanismos para desintoxicação de metais (Vijver *et al.* 2004), como armazenamento e exclusão (Posthuma & Strallen 1993, Augustianiak *et al.* 2006, Stewert *et al.* 2011). Esses mecanismos são eficientes já que invertebrados terrestres não possuem habilidade de excretar xenobióticos do sangue para o meio externo através das superfícies respiratórias (Walker & Hopkin 2006). Em nível molecular, tem sido bem documentado o efeito das metalotineínas que se ligam aos metais no corpo dos organismos, evitando seus efeitos tóxicos (Garcia-Hernandez *et al.* 1998, Hall 2002, Cobbett & Goldsbrough 2002, Janssens *et al.* 2009, Kohler *et al.* 2004, Hodson 2012).

Plantas e animais necessitam de concentrações intracelulares adequadas de íons metálicos para crescimento e desenvolvimento. Mas, o limiar de deficiência e toxicidade desses íons é diferente para cada espécie (Poschenrieder *et al.* 2006). Organismos que habitam solos ricos em metais, apesar de estarem adaptados a essas condições (Hodson 2012), sofrem os custos para metabolizar os metais, resultando em maior gasto energético e redução do fitness (Barceló & Poschenrieder 1992, Hall 2002, Augustyanik *et al*. 2006, Walker & Hopkin 2006, Maestri *et al.* 2010). Porém, é provável que eles também se beneficiem de alguma forma com o excesso de metais. Um desses benefícios tem sido mostrado para plantas que habitam solos ultramáficos (ricos em Cu, cromo - Cr, magnérsio - Mg e níquel - Ni) e solos ferruginosos (ricos em alumínio - Al, Fe, Mn e Zn), em que o acúmulo de metais nos tecidos foliares, reduz o ataque de inimigos naturais, tais como os herbívoros (Martens & Boyd 1994, Mathews *et al.* 2009, Boyd & Martens 1998, Boyd 2012, Ribeiro *et al.* 2017).

A herbivoria é uma das principais causas da redução do fitness das plantas (Coley *et al.* 1985, Coley & Barone 1996, Ribeiro & Brown 1999, Strauss *et al.* 2002, Bekaert *et al.* 2012), fato que propiciou a evolução de defesas químicas e físicas (Bernays 1981, Agrawal 2007) contra os herbívoros. A produção de compostos secundários gera um custo para as plantas já que os metabólitos destinados ao crescimento e à reprodução são desviados para vias de produção secundária (Coley 1986, Herms & Mattson 1992, Bekaert *et al.* 2012). Nesse aspecto, a defesa por acúmulo de metais - defesa elementar - é mais vantajosa porque as plantas acumuladoras detêm os metais em estruturas celulares sem que esses afetem sua fisiologia (Hossain *et al.* 2012), aumentando a eficiência e reduzindo o custo da defesa.

Se as plantas evoluíram mecanismos de tolerância e acúmulo de metais, alguns insetos herbívoros também evoluíram estratégias para lidar com os metais nas plantas. Há insetos que são capazes de evitar a toxidade dos metais por meio da dissuasão, selecionando tecidos com menor concentração de metais para ingestão. Outros, misturam alimentos de alto e baixos teores de metal, tornando a dosagem total de metais ingeridos atóxica. Há especulações de que insetos se beneficiam dos metais pesados ingeridos, usando-os como defesa contra seus predadores (Boyd & Martens 1988), mas até o fim desse trabalho, não foram encontrados estudos evidenciando esse fato. Insetos que vivem no solo e, eventualmente, usam esse recurso como abrigo e alimento, podem acumular metais em estruturas do corpo. Formigasformigas acumulam arsênio (As), cádmio (Cd), Cu, chumbo (Pb) (Toro *et al.* 2010) e selênio (Se) (De La Riva *et al.* 2014) nas mandíbulas e Cu, Cd, níquel (Ni), Mn, Pb e Zn no intestino, túbulos de Malpighi e tecidos gordurosos (Gramigni *et al.* 2013). Em algumas espécies de cupins, foi documentada a incorporação de ferro (Fe) (Yoshimura *et al.* 2002), Zn e Mn nas mandíbulas (Cribb *et al.* 2008a,b), beneficiando-os na mastigação de materiais ricos em sílica e celulose (Quicke *et al.* 1998). Há espécies de Nasutitermitinae, como *Nasutitermes*, por exemplo, que excluem partículas de Al durante a construção dos ninhos (Sarcinelli *et al.* 2009, 2013), tornando o ambiente menos tóxico que o exterior do ninho. Nessa situação, os cupins se beneficiam, mas podem beneficar também outros animais que comumente usam seus ninhos como abrigo, os coabitantes. Além disso, as condições micro-climáticas dentro do ninho são adequadas aos cupins e, consequentemente, aos seus simbiontes (coabitantes) (Holt & Lepage 2000).

Viver em solos metalíferos requer adaptações para se desintoxicar dos metais e usá-los em benefício próprio. Nesse estudo, investigamos as adaptações evolutivas de plantas e insetos em habitar solos ricos em metais, usando como modelo a espécie de planta conhecida como Candeia, *Eremanthus erythropappus* (DC.) Macleish (Asteracea) e a espécie de cupim *Cornitermes cumulans* Kollar 1832 (Insecta: Blattodea). Ambas as espécies ocorrem em áreas naturalmente enriquecidas com metais, mas que diferem entre si na concentração de metais do solo. As áreas de estudo, Parque Estadual do Itacolomi (PEIT) e Padre Viegas (PV) localizam-se em Minas Gerais, Brasil, no Quadrilátero Ferrífero (QF). O QF estende-se entre a cidade de Ouro Preto a sudeste, e Belo Horizonte, a noroeste, ao sul da Cadeia do Espinhaço (Roeser & Roeser 2010). É uma das maiores regiões produtoras de minério de ferro do mundo e abrange uma área de, aproximadamente, 7200 Km^2 e sua reserva mineral inclui Al, Fe, Mn, As, Cr, Pb e Zn (Andrade *et al.* 2012), que lhe confere uma grande importância econômica. Na canga do QF foram documentadas várias espécies vegetais que acumulam metais nos tecidos foliares em concentrações acima do disponível no substrato, tais como *Eremanthus erythopappus* (DC.) N.F.F. MacLeish, *E. glomerulatus* Less. (Asteraceae), *Microlicia crenulata* Mart., *Trembleya laniflora* Cogn. (Melastomataceae) (Teixeira & Lemos-Filho 1998), *Podocarpus sellowii* KL. (Podocarpaceae), *Schinus terebinthifolius Raddi* (Anacardiaceae), *Paepalanthus* sp. (Eriocaulaceae) e *Vellozia* sp. (Velloziaceae) (Porto & Silva 1989). Nas duas áreas dentro de estudo também ocorrem, com abundância, ninhos epígeos de cupins, principalmente da espécie *C. cumulans* (obs. pessoal).

Investigamos o efeito dos metais (Al, Cu, Fe, Mn e Zn) em insetos e plantas, considerando duas áreas com diferentes concentrações de metais nos solos. Os solos do PEIT possuem 5,6 vezes mais Al; 8,5 vezes mais Cu; 4,4 vezes mais Fe; 8 vezes mais

Mn e 21,6 vezes mais Zn que o solo de PV. Esses elementos (exceto o Al) são micronutrientes essenciais para os organismos (King 1997, Janssens *et al.* 2009), atuando em importantes funções bioquímicas e fisiológicas como participação de reações redox e composição de diversas enzimas (Coleman 1992, Epstein & Bloom 2004, Nagajyoti *et al.* 2010, Uren 2013). Porém, em altas concentrações, mesmo os metais essenciais, podem ser tóxicos para plantas (Marschner 2002, Hannam & Ohki 1988) e artrópodes (Vu *et al.* 2004, European Chemicals Agency 2008, Janssens *et al.* 2009).

Essa tese teve como objetivo geral responder a seguinte pergunta: Quais os benefícios e os custos adaptativos para plantas e insetos que se estabelecem em solos metalíferos? Elegemos a Candeia, *Eremanthus erythropappus,* para esse estudo por ser uma espécie bem adaptada a solos pobres em nutrientes e ricos em metais, acumular metais em seus tecidos (Teixeira & Lemos-Filho 1998, Ribeiro *et al*. 2017) e ser de fácil cultivo. Cupins da espécie *Cornitermes cumulans* foram os insetos estudados porque usam solo para construir seus ninhos e para retirar alimentos (Grassé 1958, Redford 1984), são encontrados em regiões onde o solo é rico em metais e seus ninhos são frequentemente invadidos por outros animais (Redford 1984, Costa *et al.* 2009, Costa & Vanin 2010).

Para responder à pergunta geral, testamos as seguintes hipóteses: (*i*) As Candeias sofrem um *trade-off* entre crescer e bioacumular ou metabolizar metais; (ii) A bioacumulação de metais por *Eremanthus erythropappus* atua como uma defesa elementar contra herbívoros mastigadores; (iii) Plantas que crescem sob o estresse dos metais e sob ataque dos herbívoros podem ter seu crescimento comprometido. A concentração de metais nos ninhos afeta: (vi) a sobrevivência dos cupins construtores e (v) a invasão dos ninhos por coabitantes.

O acúmulo de metais pelas plantas pode representar um estresse que compromete seu crescimento, mas, por outro lado, tornam-nas menos vulneráveis à herbivoria. Porém, plantas submetidas aos estresses causados pelos metais e pela herbivoria podem ter seu crescimento mais comprometido. As hipóteses *i, ii* e i*ii* são discutidas no capítulo 2. Em solos metalíferos, os invasores estariam procurando um ambiente menos tóxico para viver e os cupins, devido ao gasto energético para selecionar partículas na construção dos ninhos, estariam menos aptos a defender os ninhos. As hipóteses v*i* e *v* são discutidas no capítulo 3.

Nossos resultados mostraram que o acúmulo de metais nas folhas das Candeias não afetou significativamente a herbivoria, apesar da porcentagem de área foliar consumida ter sido extremamente baixa. Já a herbivoria afetou o crescimento das Candeias positivamente e negativamente, dependendo do conteúdo de metal (Al, Cu e Zn) nas folhas das plantas. Os cupins tiveram sua sobrevivência afetada pelo conteúdo de Zn e pela riqueza de coabitantes nos ninhos. Além disso, Zn e Mn afetaram negativamente a riqueza e a abundância de coabitantes nos ninhos.

Como os organismos colonizaram ambientes terrestres e evoluíram por milhares de anos em locais sem excesso de metais, os solos metalíferos são difíceis de serem colonizados ao longo do tempo. A colonização desses ambientes é mais complexa, lenta e custosa, já que exige adaptações fisiológicas dos organismos que os habitam. Porém, a colonização é possível e apesar dos custos, há benefícios adaptativos relacionados a estar nesses ambientes.

1.1 Referências

AGRAWAL, A. A. 2007. Macroevolution of plant defense strategies. **Trends in Ecology & Evolution**, 22(2): 103–109.

ALLOWAY, B. J. 2012. Introduction. In: Alloway BJ (ed). **Heavy metals in soils: trace metals and metalloids in soils and their bioavailability**. Environmental Pollution, Vol. 22, Springer, Dordrecht, 3–10.

ANDRADE, L. N.; LEITE, M. G. P.; BACELLAR, L. A. P. 2012. Composição mineralógica e geoquímica dos solos do parque estadual do Itacolomi - Ouro Preto/MG. **Quaternary and Environmental Geosciences,** 3(1- 2): 1–8.

ASSUNÇÃO, A. G. L.; BOOKUM, W. M.; NELISSEN, H. J. M.; VOOIJS, R.; SCHAT, H.; ERNST, W. H. O. 2003. Differential metal-specific tolerance and accumulation patterns among *Thlaspi caerulescens* populations originating from different soil types. **New Phytologist**, 159 : 411– 419.

AUGUSTYNIAK, M.; JUCHIMIUK, J. PRZYBYŁOWICZ, W. J.; MESJASZ-PRZYBYŁOWICZ, J.; BABCZYŃSKA, A.; MIGULA, P. 2006. Zinc-induced DNA damage and the distribution of metals in the brain of grasshoppers by the comet assay and micro-PIXE. **Comparative Biochemistry and Physiology, Part C,** 144: 242–251.

BAKER, D. E. 1990. Copper - chapter 8 (151-175p). In: ALLOWAY, B. J. (edit). **Heavy metal in soil.** John Wiley & Sons Inc., New York. 339p.

BAKER, A. J. M. 1981. Accumulators and excluders - strategies in the response of plants to heavy metals. **Journal of Plant Nutrition**, 3: 643–654.

BARCELÓ, J.; POSCHENRIEDER, C. 1992. Respuestas de las plantas a la contaminación por metales pesados. **Suelo y Planta**, 2(2): 345–361.

BASTA, N. T; RYAN, J. A.; CHANEY, R. L. 2005. Trace element chemistry in residual-treated soil: key concepts and metal bioavaiability. **Journal of Environmental Quality**, 34: 49–63.

BEKAERT, M; EDGER, P. P.; HUDSON, C. M.; PIRES, C.; CONANT, G. C. 2012. Metabolic and evolutionary costs of herbivory defense: systems biology of glucosinolate synthesis. **New Phytologist**, 196: 596–605.

BERNAYS, E. A. 1981. Plant tannins and insect herbivores: an appraisal. **Ecological Entomology**, 6: 353–360

BERTNESS, M. D.; CALLAWAY, R. 1994. Positive interactions in communities. **Tree** 9: 191–193.

BERTNESS, M. D.; LEONARD, G. H.; EVINE, J. M.; SCHMIDT, P. R.; INGRAHAM, A. O. 1999. Testing the relative contribution of positive and negative interactions in rocky intertidal communities. **Ecology**, 80: 2711–2726.

BLISS, L. C. 1971. Artic and Alpine plant life cycles. **Annual Review of Ecology and Systematics**, 2: 405–438.

BOHNERT, H. J.; SHEVELEVA, E. 1988. Plant stress adaptations — making metabolism move. **Physiology and Metabolism**, 267–274.

BOYD, R. S. 2012. Plant defense using toxic inorganic ions: conceptual models of the defensive enhancement and joint effects hypotheses. **Plant Science**, 195: 88–95.

BOYD, R. S.; MARTENS, S. N. 1998. The significance of metal hyperaccumulation for biotic interactions. **Chemoecology***,* 8: 1–7.

BRADL, H. B. 2005. Sources and origins of heavy metal. In: Bradl HB (edit). 2005. **Heavy metal in the environment**. *Elsevier Academic Press*, Germany. 282p.

BRADL, H. B.; KIM, C.; KRAMAR, U.; STUBEN, D. 2005. Interactions of heavy metals. In: Bradl HB (edit). 2005. **Heavy metal in the environment**. *Elsevier Academic Press*, Germany. 282p.

BROMHAM, L. 2015. Macroevolutionary patterns of salt tolerance in angiosperms. **Annals of Botany**, 115: 333–341.

CALLAWAY, R. M; BROOKER, R.W.; CHOLER, P.; KIKVIDZE, Z.; LORTIE, C. J.; MICHALET, R.; PAOLINI, L.; PUGNAIRE, F. I.; NEWINGHAM, B.; ASCHEHOUG, E. T.; ARMAS, C.; KIKODZE, D.; COOK, B. J. 2002. Positive interactions among alpine plants increase with stress. **Nature**, 417: 844–848

CLOUDSLEY-THOMPSON, J. L. 1975. Adaptation of Arthopoda to arid environmentals. **Annual Review of Entomology**, 20: 261–283.

CLOUDSLEY-THOMPSON, J. L. 2001. Thermal and water relations of desert beetles. **Naturwissenschaften**, 88:447–460

CHIARUCCI, A; ROBINSON, B. H; BONINI, I.; PETIT, D.; BROOKS, R. R.; DE DOMINICIS, V. 1998. Vegetation of tuscan ultramafic soils in relation to edaphic and physical factors. **Folia Geobotanica**, 33: 113–13.

COBBETT, C.; GOLDSBROUGH, P. 2002. Phytochelatins and metallothioneins: role in heavy metal detoxification and homeostasis. **Annual Review and Plant Biology**, 53: 159–182.

COLEY, P. D. 1986. Costs and benefits of defense by tannins in a neotropical tree. **Oecologia**, 70: 238–241

COLEY, P. D.; BARONE, J. A. 1996. Herbivory and plant defense in tropical forests. **Annual Review of Ecology and Systematics**, 27(1): 305–335.

COLEY, P. D.; BRYANT, J. B.; CHAPIM, F. S. 1985. Resource availability and plant antiherbivory defense. **Science**, 230(4728): 895–899.

COSTA, C.; VANIN, S. A. 2010. Coleoptera Larval fauna associated with termite nests (Isoptera) with emphasis on the “bioluminescent termite nests” from Central Brazil. **Psyche**, 2010: 1–12.

COSTA, D. A.; CARVALHO, R.; LIMA-FILHO, R.; BRANDÃO, D. 2009. Inquilines and invertebrate fauna associated with termite nests of *Cornitermes cumulans* (Isoptera, Termitidae) in the Emas National Park, Mineiros, Goiás, Brazil. **Sociobiology**, 53(2B): 443–453.

COLEMAN, J. E. 1992. Zinc proteins: enzymes, storage proteins, transcription factors, and replication proteins. **Annual Reviews of Biochemistry***,* 61: 897–946.

CRIBB, B. W.; STEWART, A.; HUANG, H.; TRUSS, R.; NOLLER, B.; RASCH, R.; ZALUCKI M. P. 2008a. Insect mandibles - comparative mechanical properties and links with metal incorporation. **Naturwissenschaften***,* 95: 17–23

CRIBB, B. W.; STEWART, A.; HUANG, H.; TRUSS, R.; NOLLER, B.; RASCH, R; ZALUCKI, M. P. 2008b. Unique zinc mass in mandibles separates drywood termites from other groups of termites. **Naturwissenschaften***,* 95: 433–441.

De BRUYN, L. A. L.; CONACHER, A. J. 1995. Soil modification by termites in the Central Wheatbelt of Western Australia. **Australian Journal of Soil Research***,* 33:179–193.

De La RIVA, D. G.; VINDIOLA, B. G.; CASTAÑEDA, T. N.; PARKER, D. R.; TRUMBLE, J. T. 2014. Impacts of selenium on mortality, bioacumulations and feeding deterrence in the invasive Argentine and, *Linepithema humile* (Hymenoptera: Formicidae). **Science of the Total Environment**, 481: 446–452.

DE SOUZA, O.; CRISTALDO, P. F.; ROSA, C. S.; FLORENCIO, D. F.; MARINS, A. 2012. Cupins consumindo detritos vegetais: impactos ecológicos sobre a biota tropical: 201-213. In: DEL-CLARO, K.; TORENZAN-SILINGARDI, H. M. (orgs.). **Ecologia das interações plantas-animais: uma abordagemecológico-evolutiva**. Rio de Janeiro: Technical Books. 336p.

EPSTEIN, E.; BLOOM, A. J. 2004. **Mineral nutrition of plants: principles and perspectives** (2nd ed). Sunderland: Sinauer.

ERNEST, W. H. O. 2000. Evolution of metal hyperaccumulation and phytoremediation hype. **New Phytologist**, 146: 357–358.

EVANS, L. J. 1989. Chemistry of metal retention by soils – Several processes are explained. **Environmental Science Technology**, 23(9): 1046–1056.

FADIGAS, F. S.; AMARAL-SOBRINHO, N. M. B. D. O.; MAZUR, N.; ANJOS, L. H. C., FREIXO, A. A. 2002. Concentrações naturais de metais pesados em algumas classes de solos brasileiros. **Bragantia**, 61(2): 151–159.

FLEEGER, J. W.; CARMAN, K. R.; NISBER, R. M. 2003. Indirect effects of contaminants in aquatic ecosystems. **Science of the Total Environment**, 317: 207–233.

FLOWERS, T. J.; GALAL, H. K.; BROMHAM, L. 2010. Evolution of halophytes: multiple origins of salt tolerance in land plants. **Functional Plant Biology**, 37: 604–612.

FLOWERS, T. J.; COLMER, T. D. 2015. Plant salt tolerance: adaptations in halophytes. **Annals of Botany**, 115: 327–331.

GADD, G. M. 2010. Metals, minerals and microbes: geomicrobiology and bioremediation. **Microbiology**, 156: 609–643.

GALL, J. E.; BOYD, R. S.; RAJAKARUNA, N. 2015. Transfer of heavy metals through terrestrial food webs: a review. **Environmental Monitoring and Assessment**, 187–201.

GARCIA-HERNANDEZ, M.; MURPHY, A.; TAIZ, L. 1998. Metallothioneins 1 and 2 have distinct but overlapping expression patterns in Arabidopsis. **Plant Physiology**, 118: 387–397.

GILLER, K. E.; WITTER, E.; McGRATH, S. P. 2009. Heavy metals and soil microbes. **Soil Biology and Biochemistry**, 41: 2031–2037

GRAMIGNI, E.; CALUSI, S.; GELLI, N.; GIUNTINI, L.; MASSI, M.; DELFINO, G.; CHELAZZI, G.; BARACCHI, D.; FRIZZI, F.; SANTINI, G. 2013. Ants as bioaccumulators of metals from soils: Body content and tissue-specific distribution of metals in the ant *Crematogaster scutellaris*. **European Journal of Soil Biology**, 58: 24–31.

GRASSÉ, P. P. 1958. Sur le nid et la biologie de *Cornitermes cumulans* (Kollar), termites brasilien. **Insectes Sociaux**, 5(2): 189–199.

GRENNAN, A. K. 2009. Identification of genes involved in metal transport in plants. ***Plant Physiology,*** 149: 1623–1624.

GRIME, J. P. 1997. Evidence for the existence of three primary strategies in plants and its relevance to ecologycal and evolutionary theory. **The American Naturalist**, 982: 1169–1194.

HALL, J. L. 2002. Cellular mechanisms for heavy metal detoxification and tolerance. **Journal of Experimental Botany**, 53(366): 1–11.

HANNAM, R. J.; OHKI, K. 1988. Detection of Manganese Deficiency and Toxicity in Plants (chapter 16). In: GRAHAM, R. D.; HANNAM, R. J.; UREN, N. C. (eds). **Manganese in Soils and Plants.** Developments in Plant and Soil Sciences. vol 33. Springer, Dordrecht. p. 243–259.

HE, Q.; BERTNESS, M. D.; ALTIERI, A. H. 2013.Global shifts towards positive speciesinteractions with increasing environmental stress. **Ecology Letters**,16: 695–706.

HERMS, D. A.; MATTSON, W. J. 1992. The dilema of plants: grow or defend. **The Quartlerly Review of Biology**, 67(3): 283–335.

HERNÁNDEZ, A.; Pastor, J. 2008. Relationship between plant biodiversity and heavy metal bioavailability in grasslands overlying an abandoned mine. **Environmental Geochemical and Health**, 30:127–133.

HOSSAIN, M. A.; PIYATIDA, P.; TEIXERIA DA SILVA, J. A.; FUJITA, M. 2012. Molecular mechanism of heavy metal toxicity and tolerance in plants: central role of glutathione in detoxification of reactive oxygen species and methylglyoxal and in heavy metal chelation. **Journal of Botany**, 2012: 01–37.

HODSON, M. E. 2012. Effects of heavy metals and metalloids on soil organisms. In: ALLOWAY, B. J. (ed). **Heavy metals in soils: trace metals and metalloids in soils and their bioavailability. Environmental Pollution**, Vol. 22, Springer, Dordrecht, 141–160.

HOLT, J. A.; LEPAGE, M. 2000. Termites and soil properties. In: ABE, T.; BIGNELL, D. E.; HIGASHI, M. **Termites: evolution, sociality, symbioses, ecology**. Dordrecht: Kluwer Academic Publisher. 466p.

HOPKIN, S. P.; MARTIN, M. H. 1982. The distribution of zinc, cadmium and copper within hepatopancreas of a woodlouse. **Tissue & Cell**, 14(4): 703–715.

JANSSENS, T. K. S.; ROELOFS, D.; VAN STRAALEN, N. M. 2009. Molecular mechanisms of heavy metal tolerance and evolution in invertebrates. **Insect Science**, 16: 3–18.

JARUP, L. 2003. Hazards of heavymetal contamination. **British Medical Bulletin**, 68: 167–182.

JENTSCHKE, G.; GODBOLD, D. L. 2000. Metal toxicity and ectomycorrhizas. **Physiologia Plantarum**, 109: 107–116.

JONES, D.; RAHMAN, H.; BIGNELL, D. E.; PRASETYO, A. H. 2010. Forests on ultramafic-derived soils in Borneo have very depauperate termite assemblages. **Journal of Tropical Ecology**, 26: 103–114.

JOUQUET, P.; LEPAGE, M.; VELDE, B. 2002. Termite soil preferences and particle selections: strategies related to ecological requirements. **Insectes Sociaux**, 49: 1–7.

KANDELER, F.; KAMPICHLER, C.; HORAK, O. 1996. Influence of heavy metals on the functional diversity of soil microbial communities. **Biology and Fertility of Soils**, 23(3): 299–306.

KEDDY, P. A. 1992. Assembly and response rules: two goals for predictive community ecology. **Journal of Vegetable Science**, 3: 157–164.

KING LD. 1997. Soil heavy metal. In: McLAUGHLIN, R. A. **Soil science society of North Carolina. Summary of Fortieth Annual Meeting**. Vol. XL Proceeding. 84–96.

KOHLER, A.; BLAUDEZ, D.; CHALOT, M.; MARTIN, F. 2004. Cloning and expression of multiple metallothioneins from hybrid poplar. **New Phytologist**, 164: 83–93.

KORICHEVA, J.; LARSSON, S.; HAUKIOJA, E. 1998. Insect performance on experimentally stressed woody plants: A meta-analysis. **Annual Review of Entomology**, 43: 195–216.

KRÄMER, U. 2010. Metal hyperaccumulation in plants. **Annual Review of Plant Biology**, 61: 517–534.

KRISTIJÁNSSON, J. K.; HEREGGVIDSOON, G. O. 1995. Ecology and habitats of extremophiles. **World Journal of Microbiology Biotechnology**, 11: 17–25.

LANDIS, W. G.; Y. U. M. H. 2003. **Introduction to Environmental Toxicology – impacts of chemicals upon ecological systems**. 3ed. Flórida: CRC Press.

LAVELLE, P; DECAËNS, T.; AUBERT, M.; BAROT, S.; BLOUIN, M.; BUREAU, F.; MARGERIE, P.; MORA, P.; ROSSI, J. P. 2006. Soil invertebrates and ecosystem services. **European Journal of Soil Biology**, 42: S3–S15.

LE, K. E.; WOOD, T. G. 1971. **Termites and Soils**. Academic press, London, 251 p.

LEVITT, J. 1980. **Responses of Plants to Environmental Stresses**, 2nd Edn, vol. 2. Academic Press, New York.

LUTTS, S.; LEFÈVRE, I. 2015. How can we take advantage of halophyte properties to cope with heavy metal toxicity in salt-affected areas? **Annals of Botany**, 115: 509–528.

MAESTRI, E.; MARMITOLI, M.; VISIOLI, G.; MARMIROLI, N. 2010. Metal tolerance and hyperaccumulation: costs and trade-offs between traits and environment. **Environmental and Experimental Botany**, 68: 1–13.

MARSCHNER, H. 2002. **Mineral nutrition of higher plants**. 2nd ed. Academic Press, London. 906p.

MARTENS SN, BOYD RS. 1994. The ecological significance of nickel hyperaccumulation: a plant chemical defense. *Oecologia*, 98: 379–384.

MATHEWS, S.; MA, L. Q.; RATHINASABAPATHI, C.; STAMPS, R. H. 2009. Arsenic reduced scale-insect infestation on arsenic hyperaccumulator Pteris vittata L. **Environmental and Experimental Botany**, 65: 282–286.

McPHEERSON, T. D.; MIRZA, R. S.; PYLE, G. G. 2004. Responses of wild fishes to alarm chemicals in pristine and metal-contaminated lakes. **Cannadian Journal of Zoology**, 82: 694–700.

MERTENS J, SMOLDERS E. 2012. Zinc. In: ALLOWAY, B. J. (ed). Heavy metals in soils: trace metals and metalloids in soils and their bioavailability. **Environmental Pollution**, Vol. 22, Springer, Dordrecht, 465– 493p.

NAGAJYOTI, P. C.; LEE, K. D.; SREEKANTH, T. V. M. 2010. Heavy metals, occurrence and toxicity for plants: a review. **Environmental Chemistry Letters***,* 8: 199–216.

NAJA, G. M.; VOLESKY, B. 2009. Toxicity and sources of Pb, Cd, Hg, Cr, As, and radionuclides in the environment (Chap. 2). In: WANG, L. K.; CHEN, J. P.; HUNG, Y.; SHAMAS, N. Z. (ed.). 2009. **Heavy metals in the environmental**. Boca Raton, FL: CRC Press. 492p.

NEWMAN, M. C.; UNGER, M. A. 2003. **Fundamentals of Ecotoxicology**. 2ed. Flórida: Lewis Publischers.

NOY-MEIR, I. 1973. Desert ecossystem: environmental and producers. **Annual Reviews**, 4: 25–51.

OORTS, K. 2012. Copper. In: ALLOWAY, B. J. (ed). **Heave metal in soils – trace metals and mettaloides in soils and their biovaiability**. 3 ed. Springer. New York. 613p.

POLLARD, A. J.; POWELL, K. D.; HARPER, F. A; SMITH, J. A. C. 2002. The genetic basis of metal hyperaccumulation in plants. **Critical Reviews in Plant Sciences**, 21: 539–566.

POLLARD, A. J.; REEVES, R. D.; BAKER, A. J. M. 2014. Facultative hyperaccumulation of heavy metals and metalloids. **Plant Science**, 217–218: 8–17.

PORTO, M. L.; SILVA, M. F. F. 1989.Tipos de vegetação metalófila em áreas da serra de Carajás e de Minas Gerais, Brasil. **Acta Botanica Brasilica**, 3(2):13-21.

POSCHENRIEDER, C.; TOLRA, R.; BARCELÓ, J. 2006. Can metals defend plants against biotic stress? **Trends in Plant Science**, 11 (6):288-295.

POSTHUMA, L.; STRAALEN, N. M. 1993. Heavy-metal addaptation in terrestrial invertebrates: a review of occurrence genetics, physiology and ecologial consequences. **Comparative Biochemestry and Physiology**,106(1):11-38.

PROCTOR, J. 1999. Toxins, nutrient shortages and droughts: the serpentine challenge. **Tree**, 14(9): 334-335.

QUICKE, D. L. J.; WYETH, P.; FAWKE, J. D.; BASIBUYUK, H. H.; VINCENT, J. F. V. 1998. Manganese and zinc in the ovipositors and mandibles of hymenopterous insects. **Zoological Journal of the Linean Society**, 124: 387-396.

REDOFORD, K. H. 1984. The termitaria of *Cornitermes cumulans* (Isoptera, Termitidae) and their role in determining a potential keystone species. **Biotropica**,16(2): 112–119.

RIBEIRO SP, BROWN VK. 1999. Insect herbivory in tree crowns of *Tabebuia aurea* and *T. ochracea* (Bignoniaceae): contrasting the Brazilian cerrado with the wetland Pantanal Matogrossense. *Selbyana*, 20: 159–170.

RIBEIRO, S. P.; LONDE, V.; BUENO, A. P.; BARBOSA, J. S.; CORRÊA, T. L.; SOELTL, T.; MAIA, M.; PINTO, V. D.; DUELI, G. F.; SOUSA, H. C.; KOZOVITS, A.; NALILI JR, H. A. 2017. Plant defense against leaf herbivory based on metal accumulation: examples from a tropical high altitude ecosystem. **Plant Species Biology**, 32: 147–155.

ROESER, H. M. P.; ROESER, P. A. 2010. O Quadrilátero Ferrífero – MG, Brasil: aspectos sobre sua história, seus recursos minerais e problemas ambientais relacionados. **Geonomos**, 18: 33–37.

SARCINELLI, T. S.; SCHAEFER, C. E. G. R.; LYNCH, L. S.; ARATO, H. D.; VIANA, J. H. M.; ALBUQUERQUE FILHO, M. R.; GONÇALVES, T. T. 2009. Chemical, physical and micromorphological properties of termite mounds and adjacent soils along a toposequence in Zona da Mata, Minas Gerais State, Brazil. **Catena**, 76: 107–113.

SARCINELLI, T. S.; SCHAEFER, C. E. G. R.; FERNANDES FILHO, E. I.; MAFIA, R. G.; NERI, A. V. 2013. Soil modification by termites in a sandy-soil vegetation in the Brazilian Atlantic rain forest. **Journal of Tropical Ecology***,* 29: 439–448.

SIBLY, R. M.; CALOW, P. 1989. A life-cycle theory of responses to stress. **Biological Journal of the Linnean Society**, 37: 101–116.

STEWART, A. D.; ANAND, R. R.; LAIRD, J. S.; VERRALL, M.; RYAN, C. G.; JONGE, M. D.; PATERSON, D.; HOWARD, D. L. 2011. Distribution of metals in thet ermite *Tumulitermes tumul*i (Froggatt): two types of Malpighian tubule Concretion host Zn and Ca mutually exclusively. **Plos One**, 6: 1–7.

STORK, N. E.; EGGLETON, P. 2002. Invertebrates as determinants and indicators of soil quality. **American Journal of Alternative Agriculture***,* 7(1-2): 38–47.

STRAUSS, S. Y.; RUDGERS, J. A.; LAU, J. A.; IRWIN, R. E. 2002. Direct and ecological costs of resistance to herbivory. **Trends of Ecology and Evolution**, 17(6): 278–285.

TEIXEIRA, W. A.; LEMOS-FILHO, J. P. 1998.Metais pesados em folhas de espécieslenhosas colonizadoras de uma área demineração de ferro em Itabirito, MinasGerais. **Árvore***,* 22: 381–388.

TILMAN, D. 1999. The ecological consequences of changes inbiodiversity: a search for general principles. **Ecology***,* 80: 1455–1474.

TSCHINKEL, W. R. 2003. Subterranean ant nests: trace fossils past and future? **Paleogeography Paleoclimatology Paleoecology**, 192: 321–333.

TORO, D.; FLOYD, K.; GARDEA-TORRESDEY J.; BORROK, D. 2010. Heavy metal distribution and bioacummulation in Chihuahuan Desert Rough Haverster ant (*Pogonomyrmex rugosus*) populations. **Environmental Pollution**, 158: 1281–1287.

UREN, N. C. 2013. Cobalt and manganese – chapter 12 (p.335-366). In: ALLOWAY, B. J. (ed). **Heave metal in soils – trace metals and mettaloides in soils and their biovaiability**. 3 ed. Springer. New York. 613 p.

VIJVER, M. G.; VAN GESTEL, C. A. M.; LANNO, R. P.; VAN STRAALEN, N. M.; PEIJNENBURG, W. J. G. M. 2004. Internal metal sequestration and its ecotoxicological relevance: a review. **Environmental Science and Technology***,* 38: 4705–4712.

VINCENT, R. C.; MEGURO, M. 2008. Influence of soil properties on the abundance of plant species in ferruginous rocky soils vegetation, southeastern Brazil. **Revista Brasileira de Botânica**, 31(3): 377–388.

VU, T. A.; NGUYEN, N. C.; LEADBETTER, J. R. 2004. Iron reduction in the metal-rich guts of wood-feeding termites. **Geobiology**, 2: 239–247

WAN, X.; LEI, M.; YANG, J. 2016. Two potential multi-metal hyperaccumulators found in four mining sites in Hunan Province, China. **Catena**, 148(1): 1–7.

WALKER, C. H.; HOPKIN, S. P. 2006. **Principles of Ecotoxicology**. 3ed. Londres: Taylor & Francis.

WALKER, R. B. 1954. The ecology of serpentine soil. II .Factor affecting plant growth on serpentine soils. **Ecology**, 35(2): 258–288.

YANAI, J.; ZHAO, F. J.; McGRATH, S. P.; KOSAKI, T. 2006. Effect of soil characteristics on Cd uptake by the hyperaccumulator Thlaspi caerulescens. **Environmental Pollution**, 139: 167–175.

YANG, X. H.; BROOKS, R. R.; JAFFRE, T.; LEE, J. 1985. Elemental levels and relationships in the Flacourtiaceae of New Caledonia and their significance for the evaluation of the 'serpentine problem'. **Plant and Soil**, 87: 281–291.

YOSHIMURA, T.; KAGEMORI, N.; KAWAI, S.; SERA, K.; FUTATSUGAWA, S. 2002. Trace elements in termites by PIXE analysis. **Nuclear Instruments and Methods in Physics Research B**, 189: 450–453.

Capítulo escrito de acordo com as normas da Revista *New Phytologist.*

Capítulo 2

Metal bioaccumulation reverting from negative to positive the effects of herbivores on plant growth

Dueli GF[1], DeSouza O[2], Ribeiro SP[3]

[1] Programa de Pós Graduação em Ecologia, Departamento de Biologia Geral. Universidade Federal de Viçosa, Brazil.
[2] Laboratório de Termitologia, Departamento de Entomologia, Universidade Federal de Viçosa, Viçosa, Brazil.
[3] Laboratory of Ecohealth, Ecology of Canopy Insects and Natural Sucession, Departamento de Evolução, Biodiversidade e Meio Ambiente, Universidade Federal de Ouro Preto, Ouro Preto, Brazil.

Abstract

In this study we evaluated metals accumulation effects on the growth and herbivory in a tropical tree species, *Eremanthus erythropappus* (Asteraceae). Plants were grown in a greenhouse on a soil experiment reproducing a metal concentration gradient, and lately exposed to the herbivores in natural habitat. Our results suggest that herbivory can be reverted from negative to positive type of interaction, depending on the environmental stress represented by the metal concentrations (Al, Cu and Zn). Aluminum modulated the herbivore-plant interaction negatively, with reduced plant growth rate when it was at its maximum concentration. Conversely, Cu in medium and high concentrations positively modulated the interaction, increasing the plants growth even when herbivory was high. Only a slight effect of Zn on plant growth was observed. The environmental modification promoted by the presence of metalconcentration in Candeias' leaves caused an habitat amelioration. Metals also reduce herbivores attack and low herbivory intensity promoted a compensatory effect on Candeias leading to an increase in the rate of growth. Our results show a joint action of two well described effects in the literature: habitat amelioration and the compensatory effect acting in favor of herbivore-plant interaction, modulated by metals.

Keywords: aluminun, antagonistic interactions, Candeia, compensatory effect, copper, elemental defense, habitat amelioration, plant accumulator, zinc.

2.1 Introduction

Metalliferous soils cause the evolution of plant species properly adjusted to their high level of compounds, usually toxic to plants (Pollard *et al.,* 2014). These resistant species, called metallophytes (Whiting *et al.,* 2004), are adapted to exclude, tolerate or accumulate metals in their tissues (Baker, 1981; Ernest, 2000; Pollard *et al.,* 2002). Often, the same plant may be accumulating certain metals and tolerating/excluding others, or changing its strategy according to seasonal characteristics (see Antosiewicz, 1992).

Metallophytes that accumulate high concentrations of metals in their tissues (Porto & Silva, 1989; Withing *et al.,* 2004) are called hyperaccumulators (Brooks *et al.,* 1977). Nevertheless, plants that do not fit hyperaccumulation patterns have been named only accumulators and their tissue concentrations of metals are between those of non accumulators and hyperaccumulators (Chenery, 1948; Reeves & Baker 2000; Jansen *et al.,* 2002). These criteria are not absolute but may serve as a guide to characterize metal accumulation until physiological processes are better explained (van der Ent *et al.,* 2013). Furthermore, these values may vary according to the geographic region of the plant, as demonstrated by Metali *et al.* (2012).

The reason for the evolution of metals accumulation and their supposed selective advantage is still quite controversial (Noret *et al.*, 2007). Several hypotheses have been suggested to explain this strategy, such as: (i) plant protection against pathogens and herbivores, or “elemental defense hypothesis” (Martens & Boyd, 1994); (ii) competitive advantage over non-accumulating plants by allelopathy (Reeves *et al.*, 1981; Boyd & Martens, 1998; Pollard *et al.,* 2002; Hanson *et al.*, 2003), which occurs when hyperaccumulating plants enrich the surrounding soil with metallic leaves, making the environment toxic to non-tolerant plants (Rascio & Navari-Izzo, 2011); (iii) remediation of metabolic defects, as demonstrated by Freeman *et al*. (2005) in which the Ni hyperaccumulator *Thlaspi* (Brassicaceae) reversed the hypersensitivity to pathogens; (iv) drought tolerance mechanism and (v) an accidental consequence for a more effective nutrient absorption (Vesk & Reichman, 2009). From these hypotheses 'elemental defense' is the most popular one and occurs by the death of herbivores who ingest the hyperaccumulating plant tissues (Martens & Boyd, 1994) or by the deterrent action of metals that prevent the ingesting plant tissues by herbivores (Pollard & Baker, 1997).

As herbivory is one of the causes of decreased plant fitness (Coley & Barone, 1996; Ribeiro & Brown, 1999; Strauss *et al.*, 2002), accumulating metals without affecting plant physiology (Hossain *et al.*, 2012) can be an effective and a low-cost defense strategy. Metals, unlike organic compounds, can not be degraded by herbivores (Martens & Boyd, 1994) and Noret *et al.* (2007) showed that some accumulators synthesized less secondary compounds than non-accumulating plants, reducing their energy expenditure. Costs of adapting to metalliferous soils are associated with the energy expended by the plant to detoxify the metals, migth reflect in lower growth rates (Coley *et al.* 1985; McNaughton, 1983; Strauss 1997; Mothershed & Marquis 2000; Koslov *et al.* 2012), limited energy expenditure and decreased metabolic rate (Barceló & Poschenrieder, 1992; Maestri *et al.*, 2010). Thus, minimizing herbivory rates due to metal accumulation could be an evolutionary compensation for these expenditures (Ribeiro *et al.*, 2017).

Most studies corroborate the hypothesis of elemental defense in hyperaccumulating plants (eg, Boyd & Martens, 1994; Pollard & Baker, 1997; Reeves *et al.*, 2001; Behmer *et al.*, 2003; Boyd & Jhee, 2005; Freeman *et al.*, 2007; Mathews *et al.*, 2009). However, recent studies (Coleman *et al.*, 2005; Sheirs *et al.*, 2006; Ribeiro *et al.*, 2017) have shown that low levels of accumulated metals (Cd, Mn, Ni, Pb and Zn) in plants also reduced herbivory, including these plants in the elemental defense hypothesis. Coleman *et al.* (2005) believe that the elemental defense hypothesis should be more widespread among plants than has been demonstrated and according to Boyd (2007) many elements still need be tested.

In the present study, we cultivated a metalophyte species, *Eremanthus erythropappus* DC. MacLeish (Asteraceae) in soils with different metal concentrations (Al, Cu, Fe, Mn and Zn) to test three hypotheses: (i) *E. erythropappus* suffers a conflict between bioaccumulate metals and grow; (ii) The bioaccumulation of metals by *E. erythropappus* acts as an elemental defense against chewing herbivores; and (iii) The metal accumulation in plants tissue and herbivory can affect the growth plant. Our predictions, therefore, are that metal accumulations by plants may represent a stress that compromises their growth but, on the other hand, make them less vulnerable to herbivory.

2.2 Material and methods

2.2.1 Focal species

Eremanthus erythropappus (DC.) Macleish (Asteraceae), popularly known as Candeia, is a deciduous, heliophytic and xerophytic tree with height between 6 and 10 m (Lorenzi, 2009), with some rare individuals reaching more than 15 meters emergent in mature forests (Espírito-Santo *et al.,* 2012). It has tortuous trunk, covered by thick and suberosus bark (Lorenzi, 2009), which gives the plant fire resistance. It can be found in several Brazilian states such as Minas Gerais, São Paulo, Goiás, Espírito Santo and Rio de Janeiro in high-montane or ombrophilous seasonal forests (Kamino *et al.,* 2008; Lorenzi, 2009). Candeia occurs in areas where it would be difficult to develop other species, such as shallow, poorly fertile and rocky soils at altitudes between 900 and 1800 meters (Pérez *et al.,* 2004) and on metal soils. This species has an important economic role, due to the resistance and durability of its stem and its medicinal properties. Commonly, this species forms monodominant forests called Candeial.

2.2.2 Soil collection

Soils were collected in two areas with different metal concentrations: in the Parque Estadual do Itacolomi (PEIT) and in Padre Viegas (PV). Both located in the Ferriferous Quadrangle (QF), the region with the largest iron ore production in the world.

PEIT is a Conservation Unit at a high altitude (900 to 1772 meters above sea level), located in the Ouro Preto and Mariana cities (20°22’30” – 20°30’00”S and 43°32’30” – 43°22’30”W) (Messias *et al*., 1997), at the southern portion of the Espinhaço mountain range (Peron, 1989), near the south border of the São Francisco Craton (Almeida, 1977). It occupies an area of approximately 70000 ha. Soils of the region were developed on lithological material, consisting mainly of schists, phyllites and quartzites. They are shallow (Litolics) or little to moderately deep (Cambisoils), containing primary minerals easily weathered. In these soils, high concentrations of metals such as Al, As, Ba, Cd, Co, Cr, Fe, Pb, Mn and Zn are found (Table 1 - Lista de Tabelas). This reflects local geology composed by ferruginous quartzites, canga, schists and phyllites (Andrade *et al.,* 2012).

PV is a rural area (700 metres above sea level) at Mariana city, Minas Gerais state (20°23'44.9 "S 43°20'55.5" W), 9 km away from downtown and 50 km from PEIT. The region occupies about 178 km^2 and is inserted in the lithological Nova Lima group formed by mafic-ultramafic volcanic rocks, secondary volcanic felsic rocks and sedimentary chemical and elastic rocks. These rocks contain the main gold deposits of QF (Machado *et al.,* 1996) which generated intense mineral exploration in the region in the 18th century. This region where collections were made has deep soils (Latosols) and lower concentrations of Fe, Mn, Al, Zn and Cu, compared to the PEIT soils (Table 1 - Lista de Tabelas).

2.2.3 Candeias' development

Candeias used in this experiment (n = 70) were acquired from a seedlings vivarium in tubes of 110cm^3. Seventy seedlings, initially 32 cm high on average and 6 months old, were transplanted into plastic pots with 8 liters of soil. In order to compose the pots, soils were mixed together in different proportions, constituting a concentration gradient of metals ranging from 100% of PEIT soil and 100% of PV soil to 7 levels: level 1 (0% PEIT and 100% PV), level 2 (15% PEIT and 85% PV), level 3 (30% PEIT and 70% PV), level 4 (45% PEIT and 55% PV), level 5 (60% PEIT and 40% PV), level 6 (75% PEIT and 25% PV), level 7 (100% PEIT and 0% PV) (Table 2 - Lista de Tabelas). At each level, 10 plants were grown, isolated one by one in plastic pots. Plants were kept in greenhouse at a temperature around 24 ºC and relative air humidity around 65%. The *E.*

erythropappus development was monitored in the greenhouse, by monthly measurment of plant height over 10 months.

2.2.4 Herbivory test and growth

After 10 months in a greenhouse, plants were translocated (in pots) to the PEIT, in an area dominated by a Candeial. Within this habitat, we selected five microhabitats (blocks) with different gradients of vegetation density, ranging from a more open area to a more closed area, where vegetation was denser. In each block we placed 14 plants (2 of each soil level), totaling 70 plants. Plants were monitored every 20 days during their stay in the field. At each monitoring, we counted the number of leaves with any sign of chewing on each plant, based on Ribeiro & Basset aproach (2007, 2016). The chewed leaves were marked to avoid re-counting in the next monitoring. During the experiment 2 plants were lost. After 120 days in the field, the Candeias were taken back to the greenhouse and the height of each plant was measured again. All leaves were collected and stored in paper bags. To measure the lost leaf area, we raffle and scan 20 leaves of each plant. From the images, the percentages of lost leaf area were calculated using Sigma Scan software. Approximately 5 g of leaves per plant were used to analyze metal concentrations (Al, Cu, Fe, Mn and Zn). Analyzes were performed by atomic absorption spectrophotometry by the Laboratório de Análise de Solo, Tecido Vegetal e Fertilizante of the Federal University of Viçosa - MG.

2.2.5 Statistical Analyses

Data were analysed through model selection suggested by Burnham and Anderson (2002). To do so, we used R package MuMIn in order to identify (i) the best model – the model presenting lower AIC – and (ii) all models whose AIC differs less than 2 units from the best model. We used the Second Order Information Criterion (AICc), to enhance penalty to complex models built on a limite dataset. To test hypothesis that metal accumulations affect growth plant, we created a global model to predict variable (growth) and all explanatory variables ([Al], [Cu], [Fe], [Mn], [Zn]). Global model: `growth_rate1 ~ (Al + Cu + Fe + Mn + Zn).` `growth_rate1` is the plant growth affected only by metals. That is, the Candeias' growth rate from the beginning of the experiment until before they are taken to the field; `(Al + Cu + Fe + Mn + Zn)` are metal concentration in Candeias' leaves. We found 8 candidate models, all equally probable because they do not differ from one another by more than 2 AIC units (Table 1). So, we chose the very best model, whose AICc is the lowest. The best model did not consider that none of the accumulated metals interfere with plant growth. To test hypothesis that metal accumulations affects the percentage of leaf area lost by Candeias, we created the global model to predict variable (herbivory) and all explanatory variables ([Al], [Cu], [Fe], [Mn], [Zn]): `atc_lvs ~ (Al + Cu + Fe + Mn + Zn).` `atc_lvs` is the record of herbivore attack on the leaves, measure as the number of leaves presenting any sign of herbivore attack during the 120 days of the field experiment. We found 6 candidate models, all equally probable because they do not differ from one another by more than 2 AIC units (Table 2). So, we chose the very best model, whose AICc is the lowest. The best model did not consider that none of the accumulated metals interfere with plant herbivory.

To test hypothesis that metal accumulation in plants tissue and herbivory can affect plant growth, we created a global model to predict variable (growth) and all explanatory variables ([Al], [Cu], [Fe], [Mn], [Zn]) and attacked leaves (cns_lvs). Global model: `growth_rate ~ (Al + Cu + Fe + Mn + Zn) * atc_lvs.` `growth_rate` is the growth of plants affected by metals and herbivory. It is the growth rate of the plants from the beginning to the end of the experiment, that is, after they were submitted to the herbivores in the field. We found 6 candidate models, all equally probable because they do not differ from one another by more than 2 AIC units (Table 3). So, we chose the very best model, whose AICc is the lowest. The best model hence: growth_rate ~ Al + Cu + Zn + atc_lvs + Al: atc_lvs + Cu: atc_lvs (Table 4).

2.3 Results

Withou herbivory effect, but under metal effects, the Candeias' growth was, on average 89 cm, with a minimum growth of 17 cm and a maximum of 173.5 cm. The concentration of Al, Cu, Fe, Mn and Zn in the Candeias' leaves did not affect the Candeias' growth (Table 1, Table 3 - Lista de Tabelas).

Of the 68 plants, 58 were harvested by chewers herbivorous (85.3%), but the average percentage of lost leaf area (0.95%) was extremely low. The average number of leaves attacked was with 9 leaves/plant, with a leaves attacked of 0 and a maximum of 39. The concentration of Al, Cu, Fe, Mn and Zn in the Candeias' leaves did not affect the herbivory rate (Table 2, Table 3 - Lista de Tabelas).

Under metal and herbivory effects, the Candeias' growth was, on average 89.5 cm, with a minimum growth of 18.5 cm and a maximum of 181.0 cm. The effects of herbivory on the growth of Candeias were strongly mediated by their Al, Cu and Zn foliar content (Fig. 1, Table 4). Under minimal Al content (18.7 mg/kg, green lines in all panels of figure 1), herbivores show a clear postive effect on plant growth, such an effect reverting to negative under maximal Al content (469.6 mg/Kg, red lines in all panels of figure 1). As Cu content increases (from 6.2 to 27.15 mg/Kg), green and yellow lines become steeper, indicating stronger positive effects for plants holding minimal Al content in their leaves. Such an increment in steepness was strong enough to revert the negative effects of herbivores initially observed in plants under minimal Zn content (58.4 mg/kg, yellow lines in leftmost column of panels). Copper content, however, did not succeeded in modulating herbivore effects for plants under maximal Al content. In such cases, herbivores are always deleterious to plant growth (red lines in all panels). On its turn, Zn content seem not markedly relevant for any modulation of the relationship between plants and herbivores. Its only observable effect was an mild increment of plant growth as Zn content increases, for all titres of Al and specially under low Cu content: under zero herbivore attack, plant growth is higher as Zn content increases (Fig. 1).

Table 1: Model selection table - Model with substantial empirical evidence (Δ ≤ 2) predicting metal effects in Candeias's growth cultivated in soils with different metal concentrations. Explanatory variables are heavy metal concentrations (Al, Cu, Fe, Mn, Zn). Models are based on 68 independent observations (plants) and refer to a multiple regression with generalized linear models under Gaussian and identity-link function. Int= Intercept, df = degrees of freedom used by the model, Loglik=log-likelihood, AICc = second--order Akaike information criterion, Δ = AICc difference between the model under concern and the best model, Weight = Akaike weight, that is, the likelihood of the present model being the best in the candidate set. Global model: `growth_rate_1 ~ (Al + Cu + Fe + Mn + Zn).`

Model	(Int)	Al	Cu	Fe	Mn	Zn	df	logLik	AICc	Δ	Weight
1	89.04						2	-331.43	667.0	0.00	0.21
2	71.51	0.09021					3	-330.613	667.6	0.56	0.159
18	108.70	0.13380				-0.4264	4	-329.679	668.0	0.95	0.131
26	108.10	0.14750			0.0118	-0.6021	5	-328.633	668.2	1.19	0.116
3	77.71		0.79330				3	-330.997	668.4	1.32	0.108
9	79.48				0.006736		3	-331.059	668.5	1.45	0.102
17	110.40					-0.1994	3	-331.191	668.8	1.71	0.089
5	83.17			0.01411			3	-331.231	668.8	1.79	0.086

Table 2: Model selection table - Model with substantial empirical evidence (Δ ≤ 2) predicting metal effects in Candeias's herbivory cultivated in soils with different metal concentrations. Explanatory variables are metal concentrations (Al, Cu, Fe, Mn, Zn). Models are based on 68 independent observations (plants) and refer to a multiple regression with generalized linear models under Gaussian and identity-link function. Int= Intercept, df = degrees of freedom used by the model, Loglik=log-likelihood, AICc = second-order Akaike information criterion, Δ = AICc difference between the model under concern and the best model, Weight = Akaike weight, that is, the likelihood of the present model being the best in the candidate set. Global model: `atc_lvs ~ (Al + Cu + Fe + Mn + Zn).`

Model	(Int)	Al	Cu	Fe	Mn	df	logLik	AICc	Δ	Weight
1	0.9515					2	-113.02	230.2	0.00	0.239
2	0.1795	0.003972				3	-112.04	230.4	0.23	0.213
3	0.2840		0.04673			3	-112.08	230.5	0.32	0.203
4	-0.2333	0.003289	0.03819			4	-111.43	231.5	1.27	0.126
5	0.6358			0.0007589		3	-112.66	231.7	1.48	0.114
11	0.5549		0.05991		-0.000323	4	-111.62	231.9	1.65	0.105

Table 3: Model selection table - Model with substantial empirical evidence (Δ ≤ 2) predicting metal and herbivory effects in Candeias's growth cultivated in soils with different metal concentrations. Explanatory variables include (1) metal concentrations (Al, Cu, Fe, Mn, Zn) and (2) herbivory (atc_lvs). Models are based on 68 independent observations (plants) and refer to a multiple regression with generalized linear models under Gaussian and identity-link function. Int= Intercept, df = degrees of freedom used by the model, Loglik=log-likelihood, AICc = second-order Akaike information criterion, Δ = AICc difference between the model under concern and the best model, Weight = Akaike weight, that is, the likelihood of the present model being the best in the candidate set. Global model: `growth_rate ~ (Al + Cu + Fe + Mn + Zn)* atc_lvs.`

Model	(Int)	Al	atc_lvs	Cu	Fe	Mn	Zn	Al:atc_lvs	Cu:atc_lvs	Zn:atc_lvs	df	logLik	AICc	Δ	Weight
232	46.090	-0.1354	2.380	0.06621			0.5819	-0.02443	0.1921		8	-311.977	642.4	0.00	0.238
104	20.580	-0.1318	5.838	1.66500			0.5984	-0.02740			7	-313.439	642.7	0.35	0.200
1192	-5.969	-0.2250	8.780	-0.83430			1.3010		0.2753	-0.10780	8	-312.284	643.0	0.61	0.175
1256	-4.480	-0.1580	8.952	-0.35770			1.1270	-0.01832	0.2321	-0.07453	9	-311.053	643.2	0.82	0.158
1200	-6.675	-0.1875	8.711	-1.03800	-0.02866		1.3860		0.2951	-0.11080	9	-311.255	643.6	1.22	0.129
248	49.150	-0.1239	2.028	-0.03668		0.006604	0.5050	-0.02482	0.2185		9	-311.517	644.1	1.74	0.100

Table 4: Model choosen predicting metal and herbivory effects in Candeias's growth cultivated in soils with different metal concentrations. The chosen model (232) was the one with the lowest AICc (Table 2). The minimal adequate model used to plot curves in Figure 1 was built using all terms below presenting in the very best model (232). Plotting model were, hence: `y ~ Al + Cu + Zn + atc_lvs + Al:atc_lvs + Cu:atc_lvs` for mean growth of *Eremanthus erythropappus*. Codes for variables are the same as in Table 2.

	Estimate	Std.Error	t value	Pr(>|z|)
y = mean growth of *Eremanthus erythropappus*				
(Intercept)	46.09436	32.24767	1.429	0.1580
Al	-0.13543	0.07723	-1.754	0.0845 .
Cu	0.06621	1.22475	0.054	0.9571
Zn	0.58187	0.26563	2.191	0.0323 *
atc_lvs	2.37969	2.92137	0.815	0.4185
Al:atc_lvs	-0.02443	0.01143	-2.137	0.0366 *
Cu:atc_lvs	0.19208	0.11731	1.637	0.1067

Meaning of Codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

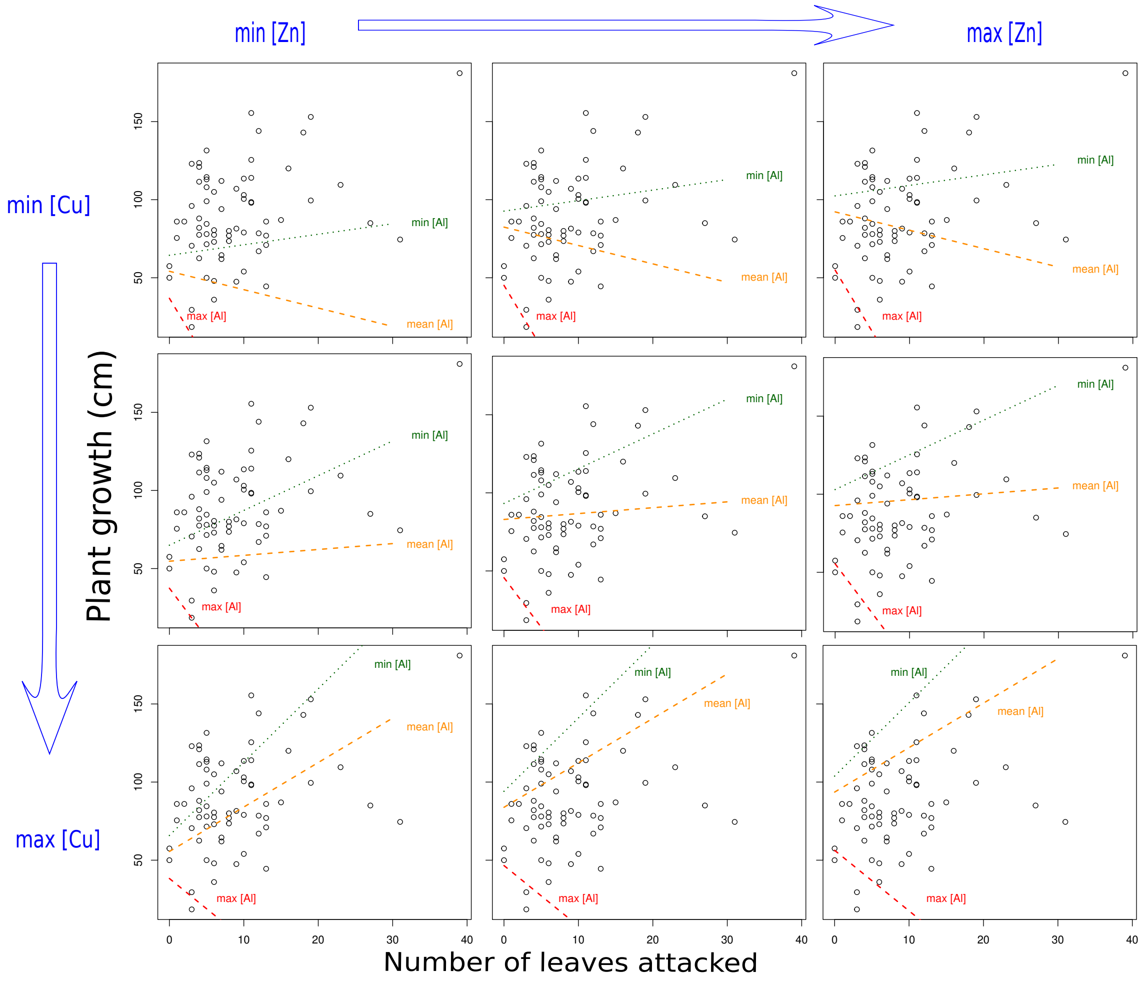

Figure 1: The effects of herbivory on the growth of Candeia as mediated by Al, Cu and Zn foliar content (mg/Kg). Leaf content in Zn and Cu increases from top left to bottom right. Curves represent minimal (min [Al]), average (mean [Al]) and maximal (max [Al]) as detected in leaves. Some curves for maximum [Al] have been realocated 20 units higher, to ease visualization.

2.4 Discussion

Our results showed that the metals do not affect the Candeias' growth and neither does it affect the herbivory and suggest that interactions classicaly considered negatives, such as herbivory, may shift to positive depending on specific ecophysiological conditions. Namely, they can be reverted to positive interactions depending on the environmental stress, here represented by metals: [Al], [Cu] and [Zn].

Regarding herbivory, the lack of detected effects here, contradicting the results observed in the same forest for other species by Ribeiro *et al.* (2017), can have a cause. The dietary constraints caused by the combination of metals and low nutritive content of these leaves in such young plants (which offer little resource in one place) would be very extreme. Thus, herbivory variance in response to experimental concentrations would not be sufficient to generate differences in averages between treatments, since herbivory was indeed very low at all concentrations tested, about 10 times lower than the global average (Koslov *et al.*, 2017; Mendes & Cornelissen, 2017). Our interpretation is that the low level of herbivory corroborate the hypothesis that there is a severe limitation towards herbivory imposed by metals accumulation, to the point that the system is prohibitive to herbivores even in the lowest concentrations studied here.

Aluminum content seems the greatest responsible for reverting herbivore effects on plants, irrespective of Zn and Cu content. Cu content seems also relevant, being able to modulate the strength herbivore effects on plant growth, at least for leaves holding minimal to medium Al content. That is, Cu content, did not succeeded in modulating herbivore effects for plants under maximal Al content. Examples of reverse interactions were presented by Bronstein (1994) showing that these interactions are not static. They can change from positive to negative (or vice versa) and fluctuate in their intensities according to the costs and benefits they provide to organisms. Our hypotheses for this result, in which the herbivore - plant interaction is modified are two effects well explained in the literature: (i) habitat amelioration and (ii) compensatory effect.

The habitat amelioration refers to environmental changes caused by physical stress (fire, tides, alternation between canopy and understory) or by living beings (neighborhood effect) that affect interactions between organisms (Bertness & Callaway, 1994; Bertness *et al*., 1999). The authors have observed habitat amelioraton affecting interactions in sessile organisms such as barnacles, mussels and terrestrial plants, generating a change in interactions outcomes from negative to positive. They support the hypothesis that the nature and strength of interspecific interactions are highly dependent on the physical environment in which they occur. Recently, Monteiro *et al.* (2017) showed that in environments with physical disturbance, ants (formerly termites predators) acted along with termites on nest defense. That is, a disturbance in the environment (fire) modified the relationship between ants and termites from predation to mutualism.

The compensatory effect has been demonstrated in plants suffering small damages by herbivores (eg, McNaughton, 1983; Vail 1992; Trumble *et al*., 1993; Stowe *et* al., 2000; Hawkes & Sullivan 2001; Poveda *et al.,* 2003). This effect may reflect better physiological responses, such as nutrient storage and higher photosynthetic rates, resulting in plant growth (Trumble *et al*., 1993; Stowe *et al*., 2000). The mechanisms behind the compensatory effect are complex and involve physiological, developmental and environmental effects (for details, see: McNaughton, 1983; Trumble *et al*., 1993; Stowe *et al*., 2000).

In this work, we suggest that metals would have caused a habitat amelioration (Bertness & Callaway, 1994; Bertness *et al.*, 1999; Monteiro *et al*., 2017) for plants, since in the absence of metals, herbivores could attack them with greater intensity. So, the metals decreased herbivores' attack capacity, and due to the low herbivory intensity, there was a compensatory effect (McNaughton, 1983), promoting Candeias' growth. We can say that the herbivore-plant relationship was modulated by metal content (Al, Cu and Zn) in Candeias' leaves, promoting a positive effect on the plants growth.

Regarding the metals that modulate the herbivore-plant interaction, we have two micronutrients used by plants: Cu and Zn, with important biochemical and physiological functions (Coleman, 1992; King 1997; Uren, 2013), and Al without known biological function (Jansen *et al.,* 2003, Gall *et al.* 2015). Copper modulated the herbivore-plant interaction positively, at least in medium and high concentrations in the leaves, promoting Candeias’ growth. Aluminum modulated this interaction negatively, especially at higher concentrations. However, the deleterious effect of Al in low and medium concentrations was suppressed by the medium and high concentrations of Cu in the Candeias’ leaves. The maximum concentrations of Cu (27.15 mg/kg) and Zn (123.8 mg/kg) in the leaves were beneficial to the plants. This may be related to the functions that these elements exert as micronutrients. However, the maximum concentration of Al in the leaves (469.6 mg/kg) was deleterious to the plants. Considering the high concentration of this metal in the soils of the two regions (PV: 15,522 mg/kg and PEIT: 87,042 mg/kg) and the role of the Candeias as Al accumulator (Teixeira & Lemos-Filho, 1998), we speculate that this metal been retained in its roots. Although these measures are not in the scope of this work, we can consider this hypothesis since studies with plants that accumulated Al in the roots (tolerant plants) had their growth compromised (Barceló & Poschenrieder, 1992; Maestri *et al.*, 2010).

The average level of herbivory found in this work was very low (0.95% or 9 consumed leaves/plant), compared to the reported for this plant species in natural conditions (2.8%) (Campos, 2012) or to other sympatric species found in forests adapted to deeper nutrient rich soils (Pereira *et al.,* 2017). And this low herbivory intensity does not rule out the possibility that the defense has occurred. However, the defense may have been so intense and the selective pressure so directional that herbivory rates showed little variation. Zinc and Copper (as sulphates), for example, are used as an insecticide with positive effects for agricultural pest control (Mogren & Trumble, 2010, review). Another issue is that even at low concentrations, metals may increase or potentiate their toxic effects on organisms when they are co-accumulated (Jhee *et al.*, 2006; Boyd, 2012). So, even though the metal concentration in Candeias trees were low in relative to hyperaccumulators plants (van der Ent *et al.*, 2013), they detained the herbivores and this seems to have occurred because of the synergistic effect between the accumulated metals.

The response of plants to herbivory is quite complex and depends on environmental, genetic, developmental stages and affected tissues (McNaughton, 1983). Our results evidenced a modification in the herbivore-plant interaction reducing the antagonism effect, showing a positive response of plants to herbivorous attack, according the stress level. We present here a new approach to herbivore-metallophytes interaction and suggest a new evolutionary possibility for the metal accumulations. We proposed the existence of an evolutionary joint mechanism of habitat amelioration and the compensatory effect, that act in favor of herbivore-plant interaction, modulated by metals. It is in this environment of tiny herbivory that the possibility arises of herbivore-plant interaction causing plant growth by some mechanism that is not yet well understood.

2.5 References

Almeida FFM. 1977. Províncias Estruturais Brasileiras. In: *SBG, Simpósio de Geologia do Nordeste, 8, Campina Grande, PB.* Atas Campina Grande 363–391.

Andrade LN, Leite MGP, Bacellar LAP. 2012. Composição mineralógica e geoquímica dos solos do parque estadual do Itacolomi - Ouro Preto/MG. *Quaternary and Environmental Geosciences* **3**: 1–8.

Antosiewicz DM. 1992. Adaptation of plants to an environmental polluted with heavy metals. Acta *Societatis Botanicor Uno Poloniae* **61**:281–299.

Baker AJM. 1981. Accumulators and excluders - strategies in the response of plants to heavy metals. *Journal of Plant Nutrition* **3**: 643–654.

Barceló J, Poschenrieder C. 1992. Respuestas de las plantas a la contaminación por metales pesados. *Suelo y Planta* **2**: 345–361.

Behmer ST, Lloyd CM, Raubenheimer D, Stewart-Clark J, Knight J, Leighton RS, Harper FA, Smith JAC. 2005. Metal hyperaccumulation in plants: mechanisms of defence against herbivores. *Functional Ecology* **19**: 55–66.

Bertness MD, Callaway R. 1994. Positive interactions in communities. *Tree* **9**: 191-193.

Bertness MD, Leonard GH, Evine JM, Schmidt PR, Ingraham AO. 1999. Testing the relative contribution of positive and negative interactions in rocky intertidal communities. *Ecology* **80**: 2711–2726.

Boyd RS. 2007. The defence hypothesis of elemental hyperaccumulation: status, challenges and new directions. *Plant and Soil* **293**: 53–176.

Boyd RS. 2012. Plant defense using toxic inorganic ions: conceptual models of the defensive enhancement and joint effects hypotheses. *Plant Science* **195**: 88–95.

Boyd RS, Jhee EM. 2005. A test of elemental defence against slugs by Ni in hyperaccumulator and non-hyperaccumulator *Streptanthus* species. *Chemoecology* **15**:179–185.

Boyd RS, Martens SN. 1994. Nickel hyperaccumulated by *Thlaspi montanum* var. *montanum* is acutely toxic to an insect herbivore. *Oikos* **70**: 21–25.

Boyd RS, Martens SN. 1998. The significance of metal hyperaccumulation for biotic interactions. *Chemoecology* **8**: 1–7.

Bronstein JL. 1994. Conditional outcomes in mutualistic interactions. *Tree* **9**(6):214-217.

Brooks RR, Lee J, Reeves RD, JaVré T. 1977. Detection of nickeliferous rocks by analysis of herbarium specimens of indicator plants. *Journal of Geochemical Exploration* **7**: 49–77.

Burnham KP, Anderson DR. 2002. *Model selection and multimodel inference: a practical information-theoretic approach*. Springer.

Campos NB. 2012. *Aptidão reprodutiva e estrutura da comunidade de um Candeial com elevada mortalidade*. M.S dissertation. Federal University of Ouro Preto, Ouro Preto, MG, Brazil.

Chenery EM. 1948. Aluminium in plants and its relation to plant pigments. *Annals of Botany* **12**: 121–136.

Coleman CM, Boyd RS, Eubanks MD. 2005. Extending the elemental defense hypothesis: dietary metal concentrations below hyperaccumulator levels could harm herbivores. *Journal of Chemical Ecology* **31**:1669–1681.

Coleman JE. 1992. Zinc proteins: enzymes, storage proteins, transcription factors, and replication proteins. *Annual Reviews of Biochemistry* **61**: 897–946.

Coley PD, Barone JA. 1996. Herbivory and plant defense in tropical forests. *Annual Review of Ecology and Systematics* **27**: 305–335.

Coley PD, Bryant JP, Chapin III FS. 1985. Resource availability and plant anti-herbivore defense. *Science* **230**: 895–899

van der Ent A, Baker AJM, Reeves RD, Pollard J, Schat H. 2013. Hyperaccumulators of metal and metalloid trace elements: Facts and fiction. *Plant and Soil* **362**: 319–334.

Ernest WHO. 2000. Evolution of metal hyperaccumulation and phytoremediation hype. *New Phytologist* **146**: 357–358.

Espírito-Santo NB, Ribeiro, SP, Lopes JFS. 2012. Evidence of competition between two canopy ant species: Is aggressive behavior innate or shaped by a competitive environment? P*syche: A Journal of Entomology (Cambridge)* **2012**:1–8.

Freeman JL, Garcia D, Kim D, Hopf A, Salt DE. 2005. Constitutively elevated salicylic acid signals glutathione-mediated nickel tolerance in *Thlaspi n*ickel hyperaccumulators. *Plant Physiolog* **137**: 1082–1091.

Freeman JL, Lindblom SD, Quinn CF, Fakra S, Marcus MA, Pilon-Smits EAH. 2007. Selenium accumulation protects plants from herbivory by Orthoptera due to toxicity and deterrence. *New Phytologist* **175**: 490–500.

Gall JE, Boyd RS, Rajakaruna N. 2015. Transfer of heavy metals through terrestrial food webs: a review. *Environmental Monitoring and Assessment*, 187-201.

Hanson B, Garifullina GF, Lindblom SD, Wangeline A, Ackley A, Kramer K, Norton AP, Lawrence CB, Pilon-Smits EAH. 2003. Selenium accumulation protects *Brassica juncea* from invertebrate herbivory and fungal infection. *New Phytologist* **159**: 461–469.

Hawkes CV, Sullivan JJ. 2001. The impact of herbivory on plants in different resource conditions:a meta-analysis. *Ecology* **82**:2045–2058

Hossain MA, Piyatida P, Teixeria da Silva JA, Fujita M. 2012. Molecular mechanism of heavy metal toxicity and tolerance in plants: central role of glutathione in detoxification of reactive oxygen species and methylglyoxal and in heavy metal chelation. *Journal of Botany* **2012**: 01–37.

Jansen S, Broadley M, Robbrecht E, Smets E. 2002. Aluminium hyperaccumulation in angiosperms: a review of its phylogenetic signifficance. *Botanical Review* **68**: 235-269.

Jansen S, Watanabe T, Dessein S, Smetes E, Robbrecht E. 2003. A comparative study of metal levels in leaves of some Al-accumulating Rubiaceae. *Annals of Botany* **91**: 657–663.

Jhee EM, Boyd RS, Eubanks MD. 2006. Effectiveness of metal-metal and metal-organic compund combinations against *Plutella xylostella*: implications for plant elemental defense. *Journal of Chemical Ecology* **32**(2):239–259.

Kamino LHY; Oliveira-Filho AT, Stehmann JR. 2008. Relações florísticas entre as fitofisionomias florestais da Cadeia do Espinhaço, Brasil, *Megadiversidade* **12**: 38–77.

King LD. 1997. Soil heavy metal. In: McLaughlin. Soil science society of North Carolina. *Summary of Fortieth Annual Meeting.* **Vol. XL Proceeding** 84–96.

Kozlov MV, Lanta V, Zverev VE, Zvereva EL. 2012. Delayed local responses of downy birch to damage by leafminers and leafrollers. *Oikos* **121**:428–434.

Koslov MV, Zverev V, Zvereva EL. 2017. Combined effects of environmental disturbance and climate warming on insect herbivory in mountain birch in subarctic forests: Results of 26-year monitoring. *Science of the Total Environment* **601–602**: 802–811.

Lorenzi H. 2009. *Árvores Brasileiras: manual de identificação e cultivo de plantas arbóreas nativas do Brasil. Volume 3.* Nova Odessa: Instituto Plantarum.

Machado N, Schrank A, Noce CM, Gauthier G. 1996. Ages of detrital zircon from Archean-Paleoproterozoic sequences: implications for Greenstone Belt setting and evolution of a Transamazonian foreland basin in Quadrilatero Ferrifero, southeast Brazil. *Earth and Planetary Science Letters* **141**: 259–276.

Maestri E, Marmitoli M, Visioli G, Marmiroli. 2010. Metal tolerance and hyperaccumulation: Costs and trade-offs between traits and environment. *Environmental and Experimental Botany* **68**: 1–13.

Martens SN, Boyd RS. 1994. The ecological significance of nickel hyperaccumulation: a plant chemical defense. *Oecologia* **98**: 379–384.

Mathews S, Ma LQ, Rathinasabapathi C, Stamps RH. 2009. Arsenic reduced scale-insect infestation on arsenic hyperaccumulator *Pteris vittata* L. *Environmental and Experimental Botany* **65:** 282–286.

McNaughton SJ. 1983. Compensatory plant growth as a response to herbivory. *Oikos* **40**: 329–336.

Mendes G, Cornelissen TG. 2017. Effects of plant quality and ant defence on herbivory rates in a neotropical ant-plant. *Ecological Entomology* **2017**: 1–8.

Messias MCTB, Sousa HC, Scalon VR, Roschel MB, Cândido ES, Fujaco MAG. 2017. Phanerogamic flora and vegetation of Itacolomi State Park, Minas Gerais, Brazil. *Biota Neotropica* **17**: 1–38.

Metali F, Salim KA, Burslem DFRP. 2012. Evidence of foliar aluminium accumulation in local, regional and global datasets of wild plants. *New Phytologist* **193**: 637–649

Mogren CL, Trumble JT. 2010. The impacts of metals and metalloids on insect behavior. *Entomologia Experimentalis et Applicata* **135**:1–17.

Monteiro I, Viana-Junior AB, Solar RRC, Neves FS, DeSouza O. 2017. Disturbance-modulated symbioses in termitophily. *Ecology and Evolution 7:10829*–10838.

Mothershead K, Marquis R. 2000. Fitness impacts of herbivory through indirect effects on plant-pollinator interactions in Oenothera macrocarpa. *Ecology* **81**:30–40.

Noret N, Meerts P, Vanhaelen M, Dos Santos A, Escarré J. 2007. Do metal-rich plants deter herbivores? A field test of the defence hypothesis. *Oecologia* **152**: 92–100.

Pereira JA, Londe V, Ribeiro SP, De Sousa HC. 2017. Crown architecture and leaf anatomic traits influencing herbivory on *Clethra scabra* Pers.: comparing adaptation to wetlands and drained habitats. *Revista brasileira de botânica* **40**: 481–490.

Pérez JFM, Scolforo JRS, Oliveira AD, Mello JM, Borges LFR, Camolesi JF. 2004. Sistema de manejo para a candeia - *Eremanthus erythropappus* (DC.) Macleish – a opção do sistema de corte seletivo. *Cerne* **10**: 257–273.

Peron MV. 1989. Listagem preliminar da flora fanerogâmica dos campos rupestres do Parque
Estadual do Itacolomi – Ouro Preto/Mariana, MG. *Rodriguésia* **67**: 63–69.

Pollard AJ, Baker AJM. 1997. Deterrence of herbivory by zinc hyperaccumulation in *Thlaspi caerulescens* (Brassicaceae). *New Phytologist* **135**: 655–658.

Pollard AJ, Powell KD, Harper FA, Smith JAC. 2002. The genetic basis of metal hyperaccumulation in plants. *Critical Reviews in Plant Sciences* **21**: 539–566.

Pollard AJ, Reeves R, Baker AJM. 2014. Facultative hyperaccumulation of heavy metals and metalloids. *Plant Science* **217– 218**: 8–17.

Porto ML, Silva MFF. 1989. Tipos de vegetação metalófila em áreas da serra de carajás e de Minas Gerais, Brasil. *Acta Botanica Brasilica* **3**: 13–21.

Poveda K, Steffan-Dewenter I, Scheu S, Tscharntke T. 2003. Effects of below- and above-ground herbivores on plant growth, flower visitation and seed set. *Oecologia* **135**:601–605.

Rascio N, F Navari-Izzo. 2011. Heavy metal hyperaccumulating plants: How and why do they do it?And what makes them so interesting? *Plant Science* **180**:169–181.

Reeves RD, Baker AJM. 2000. Metal-accumulating plants. In: Raskin I, Ensley BD, eds. *Phytoremediation of toxic metals: using plants to clean up the environment.* Wiley, New York, 193-229.

Reeves RD, Brooks RR, MacFarlane RM. 1981. Nickel Uptake by Californian *Streptanthus* and *Caulanthus* with particula reference to the hyperaccumulator *S. polygaloides* Gray (Brassicaceae). *American Journal of Botany* **68:** 708–712.

Reeves RD, Schwartz C, Morel JL, Edmondson J. 2001. Distribution and metal-accumulating behavior of *Thlaspi caerulescens* and associated metallophytes in France. *International Journal of Phytoremediation* **3**:145–172.

Ribeiro SP, Basset Y. 2007. Gall-forming and free-feeding herbivory along vertical gradients in a lowland tropical rainforest: the importance of leaf sclerophylly. *Ecography* **30**: 663-672.

Ribeiro SP, Basset Y. 2016. Effects of sclerophylly and host choice on gall densities and herbivory distribution in an Australian subtropical forest. *Austral Ecology* **441:** 219–226.

Ribeiro SP, Brown VK. 1999. Insect herbivory in tree crowns of *Tabebuia aurea* and *T. ochracea* (Bignoniaceae): contrasting the Brazilian cerrado with the wetland Pantanal Matogrossense. *Selbyana* **20**: 159–170.

Ribeiro SP, Londe V, Bueno AP, Barbosa JS, Corrêa TL, Soeltl T, Maia M, Pinto VD, Dueli GF, De Sousa HC, Kozovits A, Nalili Jr HA. 2017. Plant defense against leaf herbivory based on metal accumulation: examples from a tropical high altitude ecosystem. *Plant Species Biology* **32**:147–155.

Scheirs J, Vandevyvere I, Wollaert K, Blust R, De Bruyn L. 2006. Plant-mediated effects of heavy metal pollution on host choice of a grass miner. *Environmental Pollution* **143**:138–145.

Stowe KA, Marquis RJ, Hochwender CG, Simms EL. 2000. The evolutionary ecology of tolerance to consumer damage. *Annual Review Ecology and Systematics* **31**:565–95.

Strauss S. 1997. Floral characters link herbivores, pollinators, and plant fitness. *Ecology* **78**:1640–1645.

Strauss SY, Rudgers JA, Lau JA, Irwin RE. 2002. Direct and ecological costs of resistance to herbivory. *Trends of Ecology and Evolution* **17**: 278–285.

Teixeira WA, Lemos-Filho JP. 1998. Metais pesados em folhas de espécies lenhosas colonizadoras de uma área de mineração de ferro em Itabirito, Minas Gerais. *Revista Árvore* **22**: 381–388.

Trumble JT, Kolodnyhirsch DM, Ting IP. 1993. Plant compensation for arthropod herbivory. *Annual Review of Entomology* **38**:93–119

Uren NC. 2013. Cobalt and manganese – chapter 12 (p.335-366). In: Alloway BJ (edit). **Heavy metal in soils – trace metals and mettaloides in soils and their biovaiability.** 3 ed. Springer. New York. 613 p.

Vail SG. 1992. Selection for overcompensatory plant responses to herbivory: a mechanism for the evolution of plant-herbivory mutualism. *Ammerican Naturalist* **139**: 1-8.

Vesk PA, Reichman SM. 2009. Hyperaccumulators and herbivores – a bayesian meta-analysis of feeding choice trials, *Journal of Chemical Ecology* **35**: 289–296.

Whiting SN, Reeves RD, Richards D, Johnson MS, Cooke A, Malaisse F, Paton A, Smith AC, Angle JS, Chanei RL et al. 2004. Research priorities for conservation of metallophyte biodiversity and their Potential for Restoration and Site Remediation. *Restoration Ecology* **12**:106–116.

Capítulo 3

Termites inhabiting metalliferous soils: costs or benefits?

Dueli GF[1], DeSouza O[2], Ribeiro SP[3]

[1] Programa de Pós Graduação em Ecologia, Departamento de Biologia Geral. Universidade Federal de Viçosa, Brazil.

[2] Laboratório de Termitologia, Departamento de Entomologia, Universidade Federal de Viçosa, Viçosa, Brazil.

[3] Laboratory of Ecohealth, Ecology of tCanopy Insects and Natural Sucession, Departamento de Evolução, Biodiversidade e Meio Ambiente, Universidade Federal de Ouro Preto, Ouro Preto, Brazil.

Abstract

Metalliferous soils can be a strong habitat filter, constraining species establishment and individual survivorship, but for those evolutionarily adapted species, which may benefit from a metal rich soils. Termites are social insects that have a sophisticated relationship with the soil and theirs nests are built from soil and are complex structures, also invaded by other animals (cohabitants). In metalliferous soils, the stress of dealing with cohabitants, sum up to the further stress of metal toxicity. In this study we discuss metals (Al, Cu, Fe, Mn, Zn) effects on the *Cornitermes cumulans* survival and on the nest cohabitants species richness and abundance. We sampled 16 nests in two areas, in the southeast of Brazil, with different metal concentration. The nests had whole invertebrate species richness and abundance quantified and we collected 40 workers from each nest, for survival analysis. Our results showed a negative effect of Zn and the cohabitants richness on termite survival. Zn, along Mn affected negatively the cohabitants richness and abundance. Our results showed that there is a high cost for termites to inhabit metalliferous soils, but there are advantages in inhabitating metalliferous soils. Termites can spend more energy excluding metals in the nest construction, making them less polluted. With this, they would be weaker to defend the nest and still favor the entrance and reproduction of the cohabitants. But, if termites spend less energy in the particles selection in the nest construction, being more agile for the defense, it may affect the cohabitants entrance and reproduction. In this situation termites could be affected by metals accumulated in nests, as shown here for Zn.

Keywords: cohabitation, *Cornitermes cumulans*, manganese, survival time, termitophiles, zinc.

3.1 Introduction

Inhabit soils rich in metals (metalliferous soils) requires adaptations to deal with excess of these metals. Studies have shown plants that adapted to these environments expend significant amount of energy to metabolize metals (Barceló & Poschenrieder 1992). These plants may have their herbivory reduced by metal accumulation in their leaves (Martens & Boyd 1994, Ribeiro *et al.* 2017). Invertebrates living in these environments may ingest metals inadvertently by feeding on soils or plants, or by direct contact with metals, absorbing them through the exoskeleton or other body surface (Heikens *et al.* 2001, Jensen & Trumble 2003). These contacts must cause selective pressures which ought to promote defensive mechanisms such as avoidance, detoxification, compartmentalization, and excretion of metals (Posthuma & Strallen 1993, Augustianiak *et al.* 2006, Stewert *et al*. 2011). Insects living in metalliferous environments can accumulate metals in their body structures, as do ants (Toro *et al.* 2010, De La Riva *et al.* 2014) and termites (Fawke *et al.* 1997, Yoshimura *et al*. 2002, 2005, Crib *et al*. 2008ab). There are hypotheses on how insects benefit from the metals ingested, using them as a defense against their predators (Boyd & Martens 1988). Despite this, adaptations to these metals generates costs such as lower body mass (Rabitsch 1997), shorter life cycle, greater reproductive effort (Sibly & Calow 1989, Posthuma & Straalen 1993, Hodson 2012), increased mortality rates (Sorvari *et al*. 2006), and reduction of colony size in social insects (Eeva *et al.* 2004).

Termites (Insecta: Blattodea) are social insects that have a complex relationship with the soil. They transport clay from the deeper horizons to the surface (Schaefer 2001, Holt & Lepage 2002), changing the physico-chemical soil composition and making it more nutritious than the adjacent soil (Sarcinelli *et al.* 2009, 2013, Shaefer *et al*. 2016). In addition, most species use soil for building nests (Wood 1988) and 50% of the species feed exclusively on soil (Braumam 2000). All of these changes in the soil make the nests promote a greater biodiversity in the places where they are built and alter the availability of macro and micronutrients to the organisms, also affecting the availability and distribution of the metallic ions.

Termites occur in several biogeographical regions (Eggleton 2002). In regions enriched with metals (crome - Cr, copper - Cu, manganese - Mg, nickel - Ni, and zinc - Zn), Jones *et al*. (2010) found that termite assembly was less diverse than in other regions. Some other studies (Heikens *et al.* 2001, Bednarskca *et al.* 2013, Khan *et al.* 2017) have shown that metal concentration in the invertebrates body reflects the metal concentration in the soil. Thus, termites inhabiting mettaliferous soils are likely to have a greater deal of energy expenditure to metabolize these metals than those living in areas where such metals are scarce. Termites are able to absorb metals by though body surface (Tyler *et al*. 1989) and detoxify them by malpighi tubules (Yoshimura *et al*. 2002, Stewert *et al.* 2011).

In addition to the stress from metals, termites also must deal with the invasion of their nests by other animals - the cohabitants. They can be other termite species - called inquilines (Kalshoven 1954, Redford 1984) - or organisms belonging to other taxas, called termitophiles (Kistner 1990), which are mainly invertebrates (Brues 1932, Redford 1984, Cunha & Brandão 2000, Costa *et al*. 2009, Costa & Vanin 2010). The relationships between termites and their cohabitants are not yet well understood, but they are known to be positive (Grassé 1986) or negative (Costa & Vanin 2010, Eggleton 2011). In metalliferous regions, living within nests, where there are probably lower metal concentrations than in the external environment, could be an advantageous alternative for cohabitants to reduce the metal toxic effects.

Here we test metal effects on termite workers survival and on their cohabitants species richness and abundance. For this, we used *Cornitermes cumulans* Kollar 1832 (Termitidae: Syntermitinae) nests as a model, from two areas with different metal concentrations in the soil. Our hypotheses are: (i) metals concentrated in the nests and co-habitants richness and abundance will have negative effect on the workers survival; and (ii) metal concentration in the nests will have a positive effect on cohabitants species richness and abundance. In the construction of the nest, the workers spend energy to separate particles and in areas more enriched with metals, the workers will have greater energy expenditure in this process, becoming more debilitated. In addition, the presence of cohabitants in the nest makes the workers also spend energy defending the nest.

Therefore, metals and cohabitants are expected to have a negative effect on the survival of termites. The cohabitants richness and abundance would also be affected by the metals, since the cohabitants will have preference for nests with lower concentrations of metals.

3.2 Terms definition

"Termites" refers to all colony termites that build and inhabit the tested nests. "Nest" is the epigeic physical structure built by termites and used by them as shelter. "Builders" or "workers" are the termite caste that builds the nest, and here was used in the survival analysis experiment. "Cohabitants" or "invaders" are any invertebrate that does not belong to the colony of builders, but have ability to invade and cohabit the nest. Cohabitants include inquilines (termites of other species) and termitophiles (invertebrates in general).

3.3 Material and Methods

3.3.1 Focal species

Cornitermes cumulans is endemic to South America (Eggleton 2002) and occurs mainly in grasslands and savannas. Their nests are abundant and may reach 55 units per hectare, being considered a key species in the Brazilian Cerrado (Redford 1984). These termites feed on herbs, grasses and decomposing organic matter (Coles de Negret & Redford 1982). The nests are formed by a hypogeal and an epigeal parts (Grassé 1958). The shape is generally conical with the epigeal part reaching up to 2 meters in height and 1 meter in diameter (measured at the base) (Redford 1984). The outside wall is quite hard and formed by small irregular galleries. The central part is of black colour due to its constitution of organic matter. In addition to the rigid nest, these termites have strong mandibles, used as defence (Coles de Negret & Redford 1982), since their nests are commonly invaded by inquilines and termitophiles (Redford 1984, Costa *et al.* 2009, Costa & Vanin 2010).

3.3.2 Study area

The nests were collected in two areas: Parque Estadual do Itacolimi (PEIT), hereafter “metal rich site” and Padre Viegas (PV) district, hereafter “metal poor site”, both in the Ferriferous Quadrangle (QF), Minas Gerais State, Brazil. The QF is known worldwide for its mineral resources, mainly iron and gold. The PEIT is located between Mariana and Ouro Preto cities (20°22'30" - 20°30'00"S and 43°32'30"' - 43°22'30"W) (Messias *et al.* 1997) and occupies an area thereabout 7,000 hectares. High concentrations of metals can be found in PEIT soil such as Al, Ba, Cd, As, Cr, Fe, Hg, Mn and Zn (Table 1 - Lista de Tabelas). The nests were collected near areas of quartzites, granitic and ferruginous fields.

Padre Viegas is a district with 178 km^2 of area, located in the south-central region of the Mariana city (20°23'44.9"S 43°20'55.5"W), 9 km away from the town, and 50 Km from PEIT. The land is occupied by agriculture with pastures (natural and cropped) and eucalyptus plantations. Metals as Fe, Mn, Al and Zn and Cu (Table 1 - Lista de Tabelas) are found in their soils, but in lower concentrations than in the metal rich site. Many *C. cumulans* nests are found in farm trails or in pastures within the farms . These areas were selected due to the difference in metal concentration and presence of *C. cumulans* nests. The PEIT soils have 5.6 times more Al, 8.5 times more Cu, 4.4 times more Fe, 8.2 times more Mn and 21.6 times more Zn than the Padre Viegas soil.

These areas were selected due to the difference in heavy metal concentration and presence of *C. cumulans* nests. The PEIT soils have 5.6 times more Al, 8.5 times more Cu, 4.4 times more Fe, 8.2 times more Mn and 21.6 times more Zn than the PV soil (Table 1 - Lista de Tabelas).

3.3.3 Sampling design: nests, soils and cohabitants richness and abundance

We sampled 8 nests of *C. cumulans* in each area and to do this, we consider: (i) active nests, that is, they had termite constructors; (ii) ease access, since they would be collected whole; and (iii) possible volume to be transported, since in the regions there are nests with more than 1.8 m in height. The nests were georeferenced and had height and volume measured. Nest volume is determinant in the nest invasion: larger nests are more invaded than smaller (Cristaldo *et al.* 2012) due to diffuse soldier patrolling and thus, greater chance of enemy avoidance (DeSouza *et al*. 2016). To calculate the height, we measured the length from the base (on the ground) to the top of the nest (epigeal part). The volume was calculated by dividing the nest in slices ($F_1 + F_2 + \ldots + F_n$) together with the spherical calotte (SC), which constitutes the nest top. Considering the slice height h, the radius of the largest and smallest circumference in the slice R and r, respectively, its volume V was computed by the cone trunk equation: $V = \pi h / 3\ [R^2 + Rr + r^2]$. The volume of the spherical calotte was computed as $V = 1 / 3\pi h^2\ (3r - h)$. Then, the total volume of nests, was the sum of slices volume.

Whole nests were taken to the laboratory for sorting of cohabitants. Pieces of nests were broken into white trays, and the cohabitants were collected using tweezers or brush and kept in alcohol 80% (termitophiles) and 70% (inquilines). Termites inquilines were identified up to genus according to the taxonomic key of Constantino (2002) and the cohabitants were identified to the lowest possible taxonomic level. A portion of the soil collected from 4 samples of each nest was used for metals (Al, Cu, Fe, Mn and Zn) analysis. The metals analyzis were performed by exchangeable acidity (Al^{3+}) and atomic absorption spectrophotometry (Cu, Fe, Mn and Zn), by the Laboratório de Análise de Solos, Plantas, Tecidos e Fertilizantes of Federal University of Viçosa - MG.

3.3.4 Survival analysis

For survival analysis, we collected 40 workers from each nest (third instar and beyond). The analysis was started shortly after nests arrival in the laboratory. To minimize stress and avoid harming the workers, we collect the insects by bringing them a piece of paper; when they went up on paper, they were transferred to a petri dish. The workers were kept starved within petri dish with temperature controlled (24 to 26 ° C) according to (Costa D, unpublished paper). During the first 7 hours of experiment, termites were monitored every 30 minutes. After this period, monitoring was done every 1 hour. In each survey, the number of dead termites was recorded until 80% of workers (n = 36) were dead. The experiment was monitored for 11 hours per day.

3.3.5 Statistical analysis

The analyses were done through selection of models, using the Burnham & Anderson (2002) suggestions. We used the MuMin package to (i) identify the best model - the model with the lowest AIC - and (ii) the models whose AIC differ in less than 2 units of the best model. We use the Second order Information Criterion (AICc), which considers the sample size, increasing the relative penalty for very complex models constructed in small data sets. First, we created a global model to predict variable average time (tm) of workers survival and all explanatory variables ([Al], [Cu], [Fe], [Mn], [Zn]), richness and abundance of co-habitants and nest volume (vol). Global model: `tm ~ Al + Cu + Fe + Mn + Zn + rich + abun + vol`. (Cu), [Fe], [Mn] and [Zn]) are metal concentration in nests. We found 6 candidate models, all equally likely because they do not differ from one another by more than 2 AIC units (Table 1). In this paper, we have calculated the average model of all coefficients in all candidate models, including in the final model, which are significantly different from zero (whereas p <0.05). Following this strategy, and according to the table conditional average (Table 2), our minimum adequate model is hence: `tm ~ Zn + rich`. To test whether richness and abundance (predicted variables) were affected by metals (Al, Cu, Fe, Mn and Zn) concentration on the nest (explanatory variable), we created a global model (`rich ~ Al + Cu + Fe + Mn + Zn; abund ~ Al + Cu + Fe + Mn + Zn`). These models were subject to a generalized linear modeling (GLMs), using Poisson error distribution (log link function) corrected with Negative Binomial. Residual analyses confirmed the choice of error distribution and the suitability of the modelling equation.

3.4 Results

The nest soil contained, on average, 41 times less Cu, 3000 times less Fe, 111 times less Mn and 38 times less Zn than the soils adjacent to the nests (Tables 1 and 7 - Lista de Tabelas). We have recorded, in the 16 termitaria here studied, 2311 invertebrates of 145 morphospecies, including: Acari, Aracnidae, Coleoptera (larvae and adults), Diptera, Orthoptera, Heteroptera, Homoptera, Chilopoda, Collembola, Isopoda, Lepdoptera (larvae and pupae), Psocoptera, Pseudoescorpiones, Neuroptera, Nematoda, Dermaptera, Thysanoptera, Annelida and 27 Hymenoptera colonies, represented by 19 ant species; and 6 termite (Insecta:Blattodea: Isoptera) colonies, represented by 4 genus of Termitidae family (Tables 4, 5 and 6 - Lista de Tabelas). Data on nest measures (height and volume), concentration of metals (Al, Cu, Fe, Mn and Zn) on nest, richness and abundance of cohabitants can be seen in Table 7 (Lista de Tabelas). The mean survival time of termites depended on the Zn content in the nest and the richness of cohabitants within the nest. Both the Zn concentration and the cohabitants richness in the nest had a negative effect on the mean survival time of *C. cumulans* workers (Fig.1, Table 2). The other elements (Al, Cu, Fe and Mn) and the cohabitants abundance had not effect on the mean survival time of the workers. Coabitants richness was negatively affected by Zn ($P<0.001$) and Mn ($P<0.05$) concentration in the nest (Figs. 2 and 3). Cooper, Al and Fe did not affect coabitants richness. Coabitants abundance was negatively affected by Zn ($P<0.05$) and Mn ($P<0.001$) concentration in the nest (Figs. 4 and 5). Aluminun, Cu and Fe did not affect coabitants abundance.

Table 1: Models with substantial empirical evidence (Δ ≤ 2) predicting mean survival time of *C. cumulans* workers in two areas with different heavy metal concentrations in soil: Itacolomi State Park (PEIT) and Padre Viegas (PV), southeastern Brazil. Explanatory variables include (1) heavy metal concentrations (Al, Cu, Fe, Mn and Zn); (2) cohabitants richness (rich); (3) cohabitants abundance (abun); (4) the external volume of the termitarium (vol). Models are based on 16 independent observations and refer to a multiple regression with generalized linear models under Gaussian and identity-link function. Intrc=intercept , df = degrees of freedom used by the model, Loglik=log-likelihood, AICc=second-order Akaike information criterion, Δ=AICc difference between the model under concern and the best model, Weight = Akaike weight, that is, the likelihood of the present model being the best in the candidate set. Global model: `y ~ (Al + Cu + Fe + Mn + Zn + rich + abun + vol).`

Model	**(Intrc)**	**df**	**Loglik**	**AICc**	**Δ**	**Weight**
y = mean survival time of *C. cumulans* workers						
Zn	1444	3	-110.466	228.9	0	0.272
rich + vol + Zn	1516	5	-106.573	229.1	0.21	0.245
abun + Zn	1236	4	-109.216	230.1	1.14	0.154
Vol + Zn	1278	4	-109.52	230.7	1.74	0.114
Al + vol + Zn	1419	5	-107.351	230.7	1.77	0.112
Al + Zn	1589	4	-109.624	230.9	1.95	0.103

Table 2: Conditional average for the models predicting mean survival time of *C. cumulans* workers in two areas with different heavy metal concentrations in soil: Itacolomi State Park (PEIT) and Padre Viegas (PV), southeastern Brazil. Only models presenting ΔAICc ≤ 2 in model selection procedure (Table 2) have been included. The minimal adequate model used to plot curves in Figure 2 was built using only the terms below presenting Pr(>|z|) < .05. Nonsignificant simple terms have been kept if they took part in significant interactions. Plotting model were, hence, y ~ Zn + rich for average survival time of workers. Codes for variables are the same as in Table 2.

	Estimate	SE	Adjusted SE	z value	Pr(>|z|)
Y = mean survival time of *C. cumulans* workers					
(Intercept)	1422.562	201.784	216.532	6.57	<2e-16***
Zn	-92.667	26.215	28.297	3.27	0.0011**
rich	-18.488	7.997	8.889	2.08	0.0375*
vol	1.966	1.114	1.194	1.65	0.0996 .
abun	0.822	0.554	0.611	1.35	0.1785
Al	-463.054	309.736	339.672	1.36	0.1728

Signif. Codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

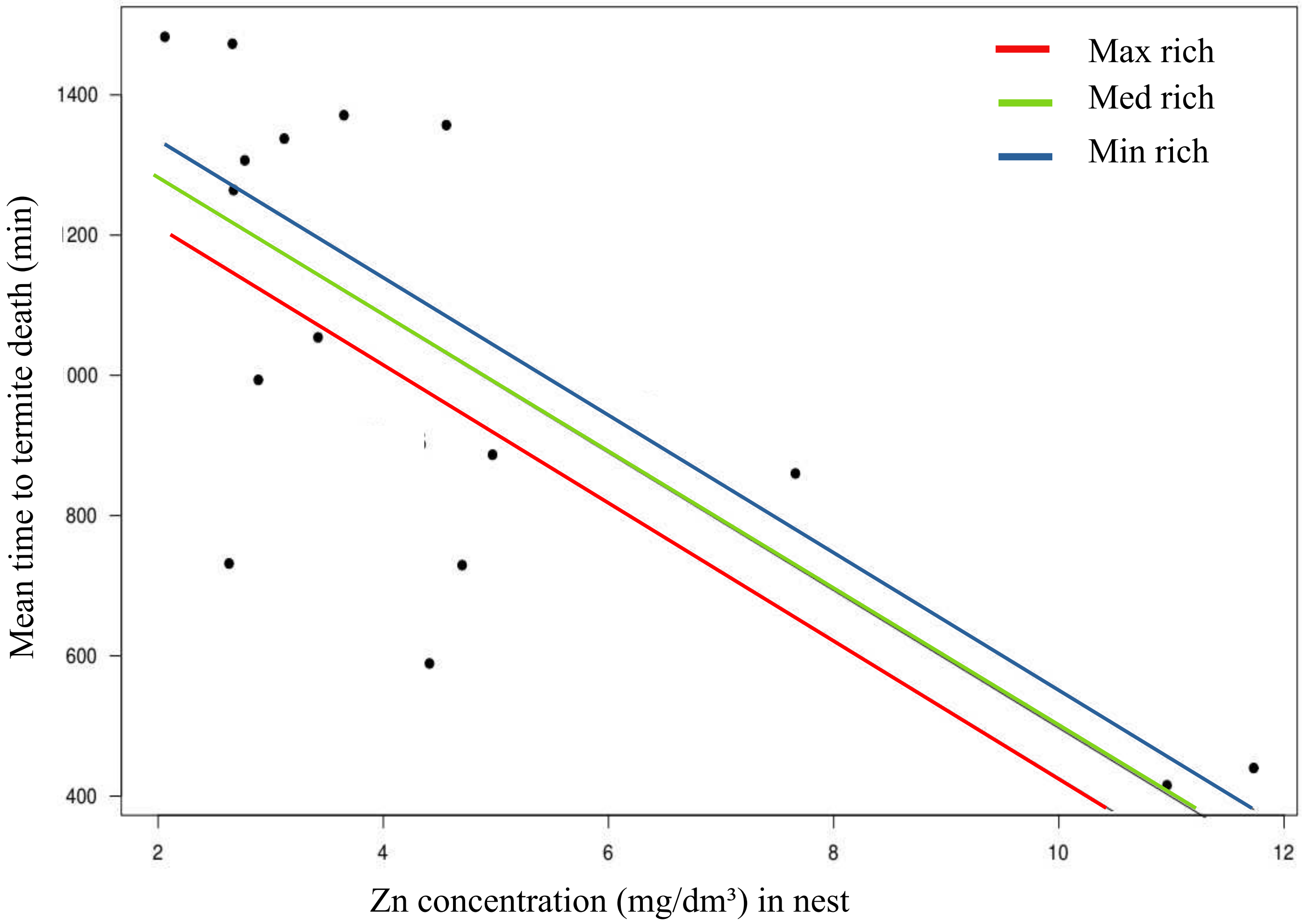

Figure 1: Effect of Zn concentration (mg/dm³) in the nest and coabitants richness in the mean survival time (minutes) of *Cornitermes cumulans* workers. Lines represent coabitants richness in the nest: maximum richness (red line, 47 coabitants), medium richness (green line, 17 coabitants) and minimum richness (blue line, 3 coabitants).

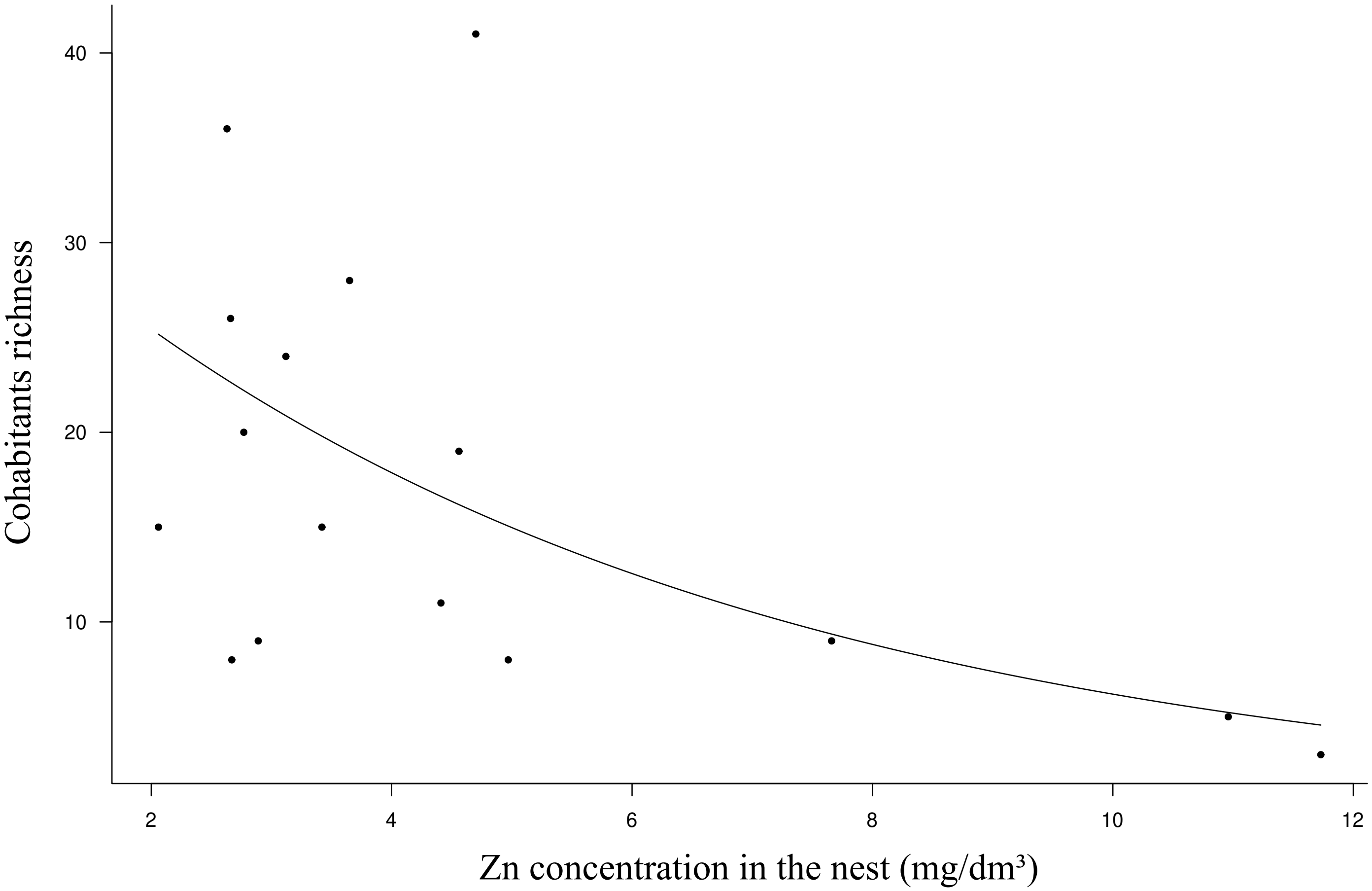

Figure 2: Effect of Zn concentration (mg/dm³) on the nests and the cohabitants richness living in the *Cornitermes cumulans* nest.
y= exp (-5.92676 -0.24623* x).

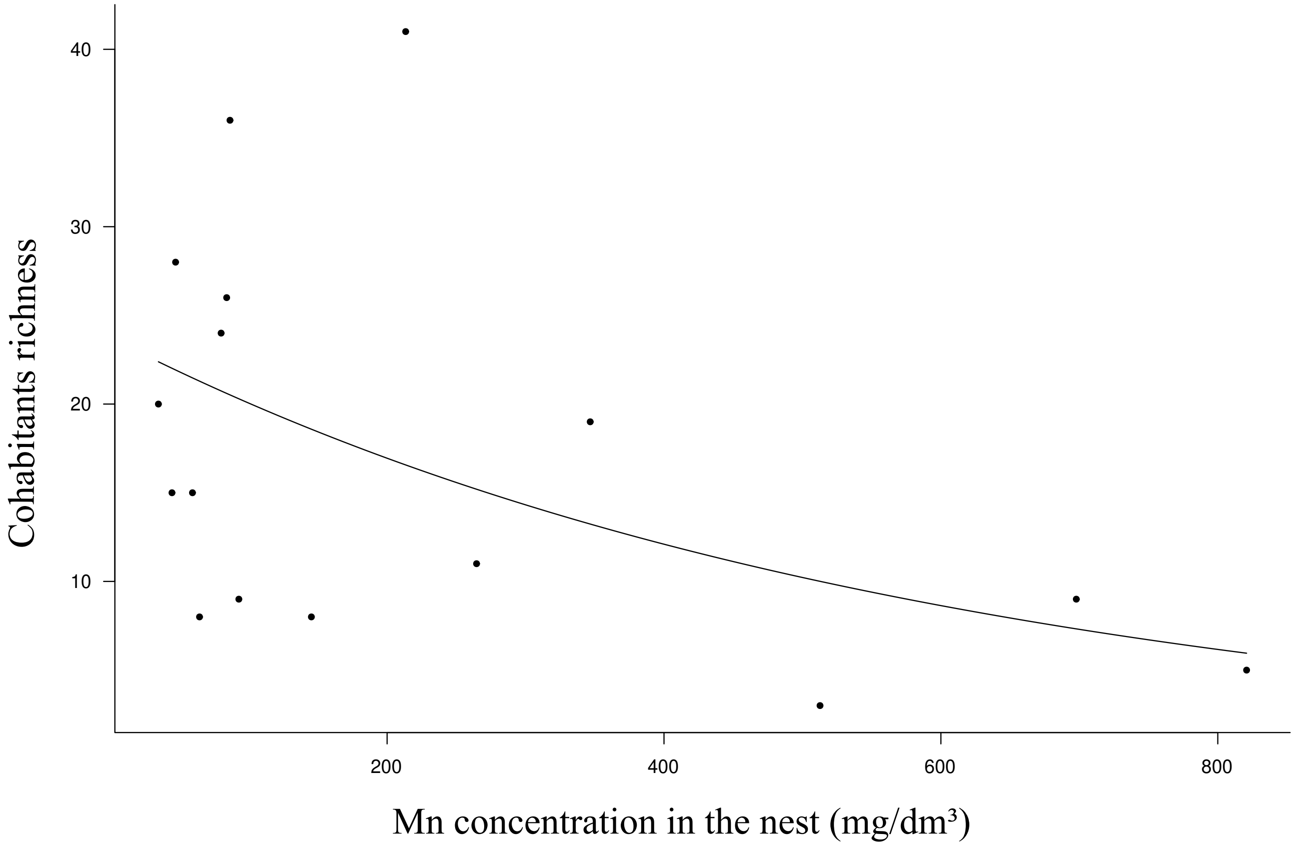

Figure 3: Effect of Mn concentration (mg_c/dm^3) on the nests and the cohabitants richness living in the *Cornitermes cumulans* nest. y= exp(3.1670005 - 0.0016849*x).

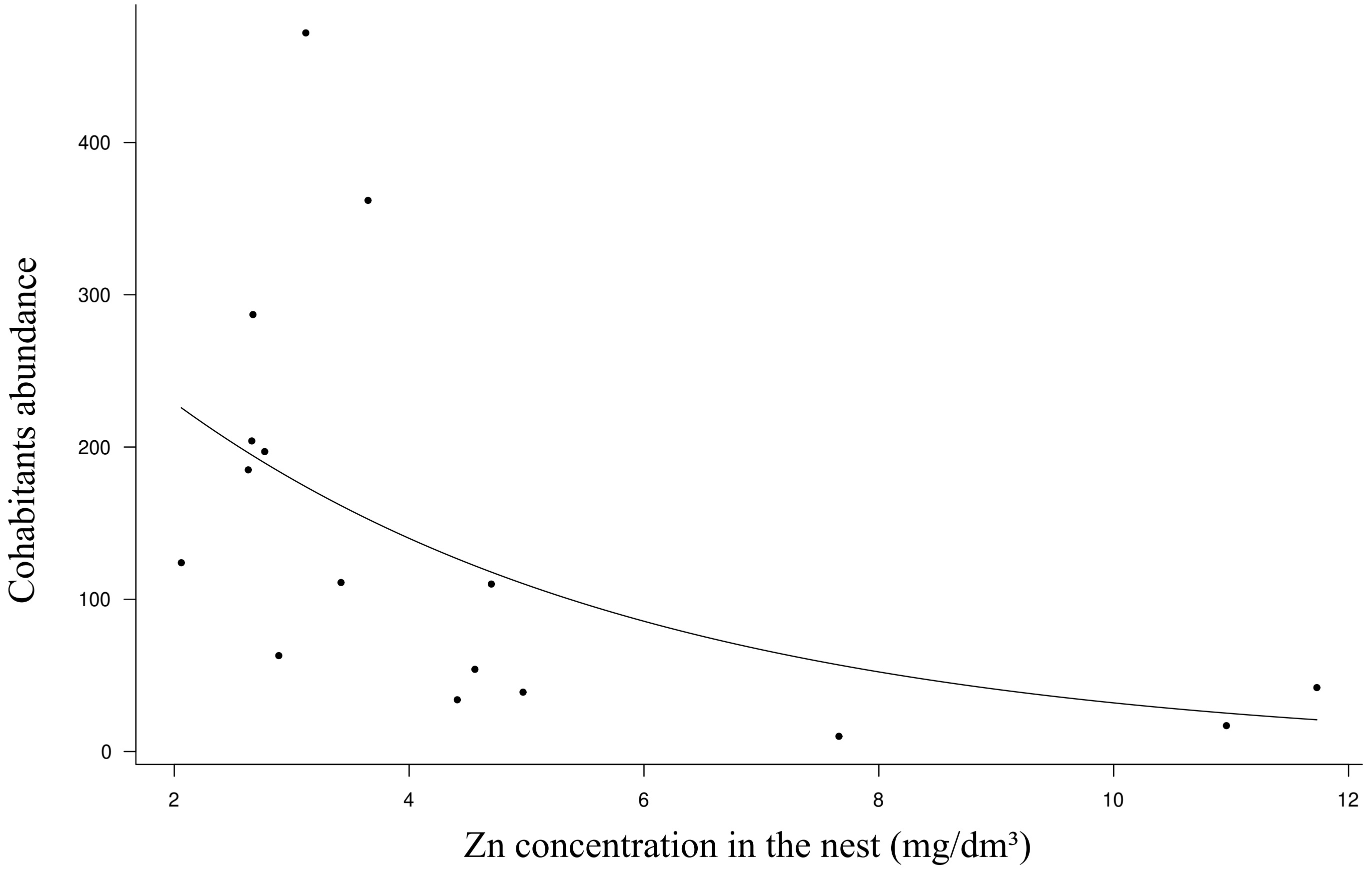

Figure 4: Effect of Zn concentration (mg/dm³) on the nests and the cohabitants abundance living in the *Cornitermes cumulans* nest. y= exp(5.92676 - 0.24623*x).

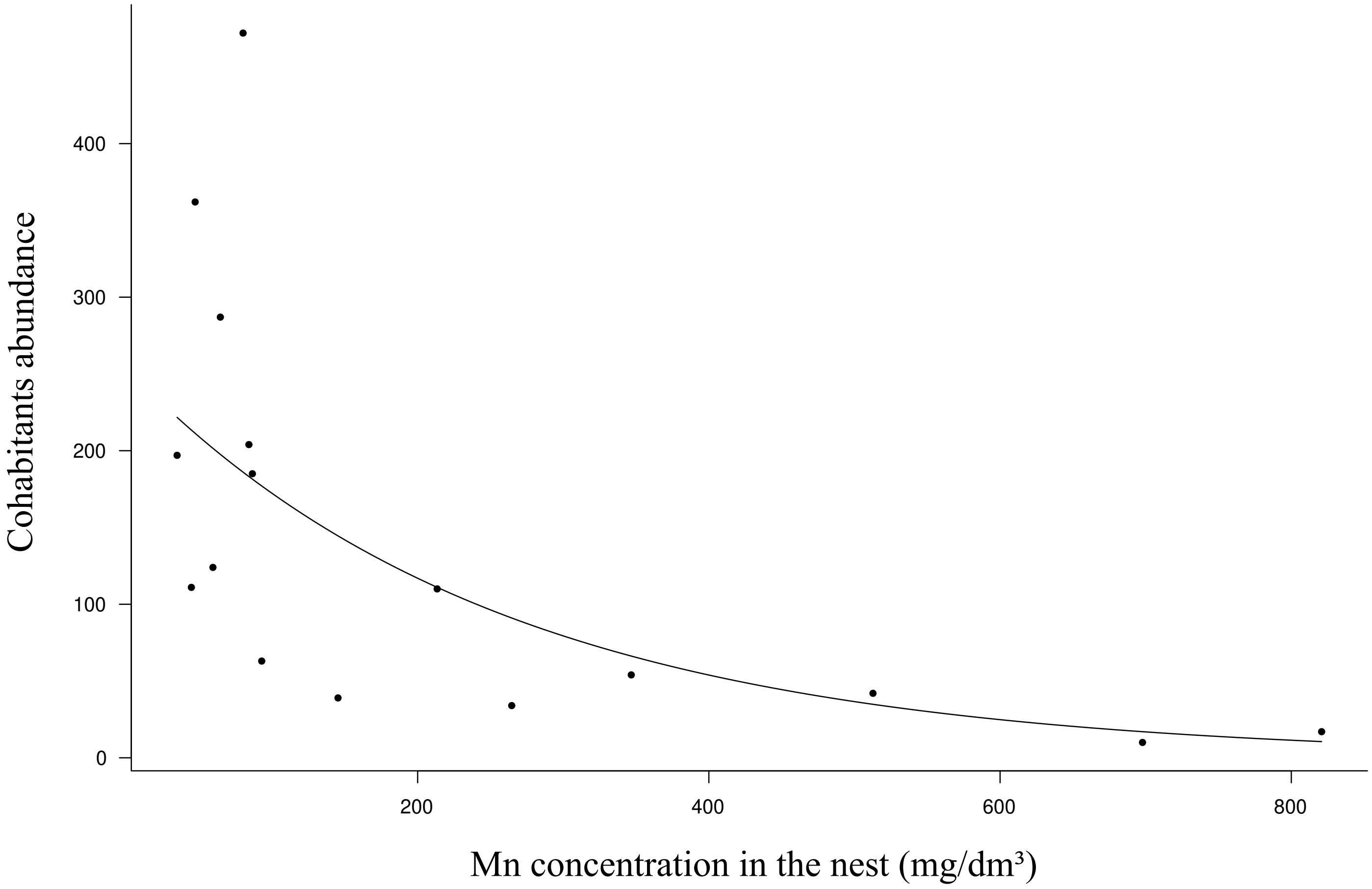

Figure 5: Effect of Mn concentration (mg_c/dm^3) on the nests and the cohabitants abundance living in the *Cornitermes cumulans* nest. y= exp(5.5361376 - 0.0038726*x).

3.5 Discussion

Our results sugest that there are costs and benefits for termites when inhabiting metalliferou soil. In one hand, the nest enriched with Zn was costly causing workers shorter lifetime. In the other, termites were benefited by Mn and Zn concentrations a reduced cohabitant populations in their nest.

Cohabitants also seems to impact negatively termite survival. However, this effect is not related to the number of individuals cohabiting the nest. That is, the effect of cohabitants is independent of their population size. This is probably because Zn and Mn affects negatively cohabitant populations in order to buffer their growth. Thus, cohabitants populations can not reach values high enough to cause some problem to the constructors. On the other hand, the Zn and Mn effects are not sufficient to completely decimate any population. Therefore, by keeping all populations low, it may allow high coexistence, and the summing up of various species populations result in high abundance of cohabitants even at high levels of Zn and Mn in the nest. For the same Zn content in the nest, the higher cohabitant richness, the lesser is the tolerance of termite builders to experimental stress (confinement in petri dishes). Hence, Zn concentration in the nest and the cohabitants richness act together, negatively affecting builders survival. It is therefore plausible to assume that a greater diversity of cohabitants may present a greater risk to the builders, making them more susceptible.

In the nests sampled, we found organisms that may have negative relationships with termites: ants (Eggleton 2011), spiders (Pekár & Toft 2014) and Elateridae (Coleoptera) larvae (Costa & Vanin 2010), are termite predators; nematodes are parasites (Rosa JMO *et al*. 2008); and Scarabaeidae larvae, depending on the nest resources (Costa & Vanin 2010), compete with the builders. Stress caused by these interactions can affect builder survival, as there is an energy demand to defend the nest against predators, compete for resources and tolerate parasites. In nests with a greater cohabitant's richness, termites are likely to suffer more from negative interactions. In nests that have only parasites, there is only one kind of stress compared to nests where predators, competitors and parasites coexist.

The first barrier encountered by the invaders when trying to enter *C. cumulans* nest is the nest itself, formed by very rigid walls. There are also the soldiers, with their chemical and physical defences (Prestwich 1984) and workers who rebuild damaged parts of the nest (Coles de Negret & Redford 1982). In soils enriched with metals, termites may spend more energy on nests building, since they would have to select particles to avoid accumulating metals on the walls of nests, as demonstrated by Sarcinelli *et al.* (2009, 2013) for Al. In our study, the nest soil contained much less concentrated metals than the soils adjacent to the nests. This means that termites have selected particles to build the nests, and this energy expenditure may have made them weaker. Thus, it is justified the faster death of workers during the experiment (confinement in petri dishes) in nests with higher Zn concentrations. This energy expenditure may also have facilitated the entrance of the cohabitants, since the builders would be less able to defend the nest.

Cohabitants richness was negatively affected by Zn and Mn concentrations in the nests and the cohabitants could choose between nests of the same area, those with lower metal concentrations Therefore, the fact that nests with lower metal concentrations have a greater cohabitant richness can be explained by two reasons: (i) nests with lower metal concentrations are those in which the workers spent more energy to keep them less toxic and therefore are less adept at defending, facilitating the entry of invaders (cohabitants); (ii) in the same area, invaders would be more willing to invade nests with lower metal concentrations because they prefer less toxic environments and in this case would find an easier defense to be welshed.

Manganese and Zn also negatively affected the cohabitants abundance. In this case, we can infer that these metals effects on cohabitant populations may be related to the energy expended to detoxify from metals excess. Studies have shown that the detoxification process can affect the reproduction of organisms coping with metals in the environment (Sibly & Calow 1989, Posthuma & Straalen 1993, Walker & Hopkin 2006, Hodson 2012). Thus, in nests with higher metals concentration, the organisms (cohabitants) had greater energy expenditure to detoxify these metals, reflecting in reduction of the reproductive capacity, that is, lower abundance.

Our results showed that there is a high cost for termites to inhabit metalliferous soils, but there are advantages in inhabitating metalliferous soils. The lower metal concentrations in the nests relative to the adjacent soil suggest that there was a work of particles selection to construct the nest, resulting in less energy for the builders to defend the nest. That is, termites that inhabit more enriched soils with metals spend more energy in the construction of the nests in relation to the termites that inhabit soils with less metals. With the exception of Zn, the other metals did not directly affect the survival of the workers, but may have contributed indirectly to this process since there is an extra energy expenditure to select particles in the construction of the nest. On the other hand, the same metal that negatively affects workers' survival (Zn), along to Mn benefits termites by adversely affecting the cohabitants richness and abundance in nests.

It seems that there is a dilemma for termites in selecting or not the metal particles in nests construction: (i) to spend more energy excluding metals in the nest construction, making them less polluted than neighboring nests. With this, they would be weaker to defend the nest and still favor the entrance and reproduction of the cohabitants; or (ii) to spend less energy in the particles selection in the nest construction, being more agile for the defense and, consequently, disfavoring the cohabitants entrance and reproduction. But, in this situation termites could be affected by metals accumulated in nests, as shown here for Zn.

3.6 References

Ahsanullah M. 1976. Acute toxicity of cadmium and zinc to seven invertebrate species from Western Port, Victoria. *Australian Journal of Marine and Freshwater Research,* 27:187-196.

Augustyniak M, Juchimiuk J, Przybyłowicz WJ, Mesjasz-Przybyłowicz J, Babczyńska A, Migula P. 2006. Zinc-induced DNA damage and the distribution of metals in the brain of grasshoppers by the comet assay and micro-PIXE. *Comparative Biochemistry and Physiology, Part C, 144:*242-251.

Barceló J, Poschenrieder C. 1992. Respuestas de las plantas a la contaminación por metales pesados. *Suelo y Planta,* 2: 345–361.

Bednarskca AJ, Stachowicz I, Kuriańska L. 2013. Energy reserves and accumulation of metals in the ground beetle *Pterostichus oblongopunctatus* from two metal-polluted gradients. *Environmental Science Pollution Research*, 20:390–398.

Boyd RS, Martens SN. 1998. The significance of metal hyperaccumulation for biotic interactions. *Chemoecology*, 8:1–7.

Brauman A. 2000. Effect of gut transit and mound deposit on soil organic matter transformations in the soil feeding termite: A review. *European Journal of Soil Biology*, 36: 1-9.

Brues CT. 1932. Phoridae associated with ants and termites in Trinidad. *Psyche,* 39, 134–138.

Burnham KP, Anderson DR. 2002. *Model selection and multimodel inference: a practical information-theoretic approach*. Springer.

Coles de Negret HR, Redford KH. 1982. The biology of nine termite especies (Isoptera: Termitidae) from the Cerrado of Central Brazil. *Psyche*, 89: 81-106.

Coles H. 1980. Defensive strategies in the ecology of neotropical termites. PhD Thesis - Southampton University, U.K. 243pp.1-2p.

Constantino R. 2012. On-line Termites Database. Avaiable in <http://http://164.41.140.9/catal/> Acessed in July, 30, 2015.

Costa C, Vanin SA. 2010. Coleoptera Larval fauna associated with termite nests (Isoptera) with emphasis on the “bioluminescent termite nests” from Central Brazil. *Psyche*, 2010:1-12.

Costa DA, Carvalho R, Lima-Filho R, Brandão D. 2009. Inquilines and invertebrate fauna associated with termite nests of *Cornitermes cumulans* (Isoptera, Termitidae) in the Emas National Park, Mineiros, Goiás, Brazil. Sociobiology, 53(2B): 443–453.

Cribb BW, Stewart A, Huang H, Truss R, Noller B, Rasch R, Zalucki MP. 2008a. Insect mandibles - comparative mechanical properties and links with metal incorporation. *Naturwissenschaften,* 95:17–23

Cribb BW, Stewart A, Huang H, Truss R, Noller B, Rasch R, Zalucki MP. 2008b. Unique zinc mass in mandibles separates drywood termites from other groups of termites. *Naturwissenschaften,* 95: 433-441.

Cristaldo PF, Rosa C S, Florencio DF, Marins A, DeSouza O. 2012. Termitarium volume as a determinant of invasion by obligatory termitophiles and inquilines in the nests of *Constrictotermes cyphergaster* (Termitidae, Nasutitermitinae). *Insectes Sociaux,* 59:541-548.

Cunha HF, Costa DA, Santo-Filho K, Brandão D. 2003. Relationship between *Constrictotermes cyphergaste*r and inquiline termites in the Cerrado (Isoptera: Termitidae). *Sociobiology,* 42: 761-770.

Cunha HF, Brandão D. 2000. Invertebrates associated with the Neotropical termite *Constrictotermes cyphergaster* (Isoptera: Termitidae, Nasutitermitinae). *Sociobiology*, 37:593–599.

De La Riva DG, Vindiola BG, Castañeda TN, Parker DR, Trumble JT. 2014. Impacts of selenium on mortality, bioacumulations and feeding deterrence in the invasive Argentine and, *Linepithema humile* (Hymenoptera: Formicidae). *Science of the Total Environment*, 481:446-452.

DeSouza O, Araújo APA, Florencio DF, Rosa CS, Marins A, Costa DA, Rodrigues VB, Cristaldo PF. 2016. Allometric scaling of patrolling rate and nest volume in

Constrictotermes cyphergaster termites: hints on the settlement of inquilines. *Plos One*, 11(1):1-13. e0147594. doi:10.1371/journal.pone.0147594

Eeva T, Sorvari J, Koivunen V. 2004. Effects of heavy metal pollution on red wood ant (*Formica* s. str.) populations. *Environmental Pollution*, 132: 533–539.

Eggleton P. 2002. Global patterns of termite diversity. In: Abe T, Bignell DE, Higashi M (eds.) *Termites: evolution, sociality, symbioses, ecology*. Dordrecht: Kluwer Academic Publisher. 466p.

Eggleton P. 2011. An introduction to termites: biology, taxonomy and functional morphology. In: Bignell DE, Roisin Y, Lo N (eds.), *Biology of termites: a modern synthesis.* Springer-Verlag, Berlin. 513 p.

Fawke JD, McClements JG, Wyeth P. 1997. Cuticular metals: quantification and mapping by complementary techniques. *Cell Biology International*, 21:675-678.

Fujaco MAG, Leite MGP, Messias MCTB. 2010. Análise multitemporal das mudanças no uso e ocupação do Parque Estadual do Itacolomi (MG) através de técnicas de geoprocessamento. *Revista Escola de Minas,* 63: 695- 701.

Grassé PP. 1958. Sur le nid et la biologie de *Cornitermes cumulans* (Kollar), termites brasilien. *Insectes Sociaox*, 5(2):189-199.

Grassé PP. 1986. *Termitologia, vol. III*. Masson, Paris - New York.
Heikens A, Peijnenburg WJGM, Hendriks AJ. 2001. Bioaccumulation of heavy metals in terrestrial invertebrates. *Environmental Pollution*, 113:385–393.

Hodson ME. 2012. Effects of heavy Metals and Metalloids on soil organisms. In: Alloway BJ (ed). *Heavy metals in soils: trace metals and metalloids in soils and their bioavailability*. Environmental Pollution, Vol. 22, Springer, Dordrecht, 141-160.

Holt JA, Lepage M. 2002. Termites and soil properties. In: Abe T, Bignell DE, Higashi M. Termites: evolution, sociality, symbioses, ecology. Dordrecht: Kluwer Academic Publisher. 466p.

Jacobi CM, Carmo FF. 2008. The contribution of ironstone outcrops to plant diversity in the Iron Quadrangle, a threatened Brazilian landscape. *AMBIO*, 37(4):324-326.

Jensen P, Trumble JT. 2003. Ecological consequences of bioavaiability of metals and metalloids in insects. *Recent Research Developtment in Entomology*, 4:1-17.

Jones D, Rahman H, Bignell DE, Prasetyo AH. 2010. Forests on ultramafic-derived soils in Borneo have very depauperate termite assemblages. *Journal of Tropical Ecology*, 26:103–114.

Kalshoven LGE. 1954. On the change in occupation by termite colonies of mounds after conversion of a jungle area into rice-fields in Java. *Insectes Sociaux*, 1:319–323.

Khan SR, Singh SK, Rastogi N. 2017. Heavy metal accumulation and ecosystem engineering by two common mine site-nesting ant species: implications for pollution-level assessment and bioremediation of coal mine soil. *Environmental and Monitoring Assessment,* 189-195.

Kistner DH. 1990. The integration of foreign insects into termite societies or why do termites tolerate foreign insects in their societies? *Sociobiology,* 17: 191-215.

Martens SN, Boyd RS. 1994. The ecological significance of nickel hyperaccumulation: a plant chemical defense. *Oecologia* 98: 379–384.

Messias MCTB, Souza HC, Scalon VR, Roschel MB, Cândido ES, Fujaco MAG. 2017. Phanerogamic flora and vegetation of Itacolomi State Park, Minas Gerais, Brazil. *Biota Neotropica,* 17(1): e20160236

Oliveira OAB, Olivito JPR, Rodrigues-Silva D. 2011. Caracterização da Unidade Espeleológica e das Unidades Geomorfológicas da Região do Quadrilátero Ferrífero – MG. Espeleo-Tema, 22(1): 61-80.

Pekár S, Toft S. 2014. Trophic specialisation in a predatory group: the case of prey-specialised spiders (Araneae). *Biological Reviews*, 90(3), 744–761.

Posthuma L, Straalen NM. 1993. Heavy-metal addaptation in terrestrial invertebrates: a review of occurrence genetics, physiology and ecologial consequences. *Comparative Biochemestry and Physiology*,106(1):11-38.

Prestwich G. 1984. Defense mechanisms of termites. *Annual Review Entomology*, 29:201–232.

Rabitsch WB. 1997. Seasonal metal accumulation patterns in the red wood ant *Formica pratensis* (Hymenoptera) at contaminated and reference sites. *Journal of Applied Ecology,* 34: 1455–1461.

Redoford KH.1984. The termitaria of *Cornitermes cumulans* (Isoptera, Termitidae) and their role in determining a potential keystone species. *Biotropica*,16(2): 112-119.

Ribeiro SP, Londe V, Bueno AP, Barbosa JS, Corrêa TL, Soeltl T, Maia M, Pinto VD, Dueli GF, Sousa HC, Kozovits A, Nalili Jr HA. 2017. Plant defense against leaf herbivory based on metal accumulation: examples from a tropical high altitude ecosystem. *Plant Species Biology*, 32:147–155.

Rosa CS, Marins A, DeSouza O. 2008. Interactions between beetle larvae and their termite hosts (Coleoptera; Isoptera, Nasutitermitinae). *Sociobiology*, 51:191-197.

Rosa JMO, Wilcken SRS, Wilcken CF, Leite LG. 2008. Patogenicidade de *Steinernema carpocapsae* (Rhabditida: Steinernematidae) ao cupim de montículo *Cornitermes cumulans* (Isoptera:Termitidae). *Nematologia Brasileira*, 32: 260-269.

Sarcinelli TS, Schaefer CEGR, Lynch, Arato HD, Viana JHM, Albuquerque Filho MR, Gonçalves TT. 2009. Chemical, physical and micromorphological properties of termite mounds and adjacent soils along a toposequence in Zona da Mata, Minas Gerais State, Brazil. *Catena*, 76:107-113.

Sarcinelli TS, Schaefer CEGR, FernandesFilho EI, Mafia RG, Neri AV. 2013. Soil modification by termites in a sandy-soil vegetation in the Brazilian Atlantic rain forest. *Journal of Tropical Ecology,* 29:439–448.

Schaefer CER. 2001. Brazilian latosols and their B horizon microstructures as long-term biotic constructs. *Australian Journal of Soil Research,* 39: 909–929.

Schaefer CEGR, Marins A, Corrêa GR, DeSouza, Nunes JA. 2016. *Chapter 16 - Termite role in soil nutrient cycling in ironstone rupestrian grasslands (canga) in Carajás, Brazilian Amazonia.* 379-390p. In: Fernandes WG (ed.). Ecology and conservation in mountaintop grasslands in Brazil. 567p.

Sibly RM, Calow P. 1989. A life-cycle theory of responses to stress. *Biological Journal of the Linnean Society*, 37:101–116.

Sorvari J, Rantala LM, Rantala MJ, Hakkarainen H, Eeva T. 2006. Heavy metal pollution disturbs immune response in wild ant populations. *Environmental Pollution,* 145(1):324–328.

Souza LA, Sobreira FG, Prado-Filho JF. 2005. Cartografia e diagnóstico geoambiental aplicados ao ordenamento territorial do município de Mariana – MG. *Revista Brasileira de Cartografia*, 57(03): 189 – 203.

Stewart AD, Anand RR, Laird JS, Verrall M, Ryan CG, Jonge MD, Paterson D, Howard DL. 2011. Distribution of metals in thet ermite *Tumulitermes tumul*i (Froggatt): two types of Malpighian tubule Concretion host Zn and Ca mutually exclusively. *Plos One*, 6:1-7.

Stewart AD, Anand RR, Balkau J. 2012. Source of anomalous gold concentrations in termite nests, Moolart Well, Western Australia: implications for exploration. *Geochemistry: Exploration, Environment, Analysis*, 12: 327 –337.

Toro D, Floyd K. Gardea-Torresdey, Borrok D. 2010. Heavy metal distribution and bioacummulation in Chihuahuan Desert Rough Haverster ant (*Pogonomyrmex rugosus*) populations. *Environmental Pollution*, 158:1281-1287.

Tyler G, Balsberg Pahllson, Bengtsson G, Baath E, Tranvik L. 1989. Heavy metal ecology on terrestrial plants, microorganisms and invertebrates - a Review. *Water, Air, and Soil Pollution,* 47: 189-215.

Walker CH, Hopkin SP. 2006. *Principles of Ecotoxicology*. 3ed. Londres: Taylor & Francis.

Wood TG. 1988. Termites in the soil environmental. *Biology and Fertility of Soils*, 6: 228-236.

Yoshimura T, Kagemori N, Kawai S, Sera K, Futatsugawa S. 2002. Trace elements in termites by PIXE analysis. *Nuclear Instruments and Methods in Physics Research B,* 189:450–453.

Yoshimura T, Kagemori N, Sugiyama J, Kawai S, Sera K, Futatsugawa S, Yukawa M, Imazeki H. 2005 Elemental analysis of worker mandibles of *Coptotermes formosanus* (Isoptera: Rhinotermitidae). *Sociobiology,* 45:255-259.

Capítulo 4

Conclusões Gerais

Nesse trabalho avaliamos os custos e os benefícios para plantas e insetos habitarem solos metalíferos. O baixo nível de herbivoria nas Candeias, comparado ao encontrado para essa espécie, sugere que os metais tenham atuado reduzindo o ataque de herbívoros (defesa elementar), beneficiando as plantas. Além disso, os metais nas folhas modularam a interação herbívoro-planta apontando para uma mudança no papel dos herbívoros de negativo para positivo, desde que as taxas de herbivoria sejam baixas. O estudo com cupins corroborou com nossas hipóteses, mostrando que há uma relação custo-benefício para esses organimos em habitar solos metalíferos. Apesar do Zn ter afetado a sobrevivência dos cupins, o metal, juntamente com o Mn, os beneficiou tamponando o crescimento das populações de coabitantes.

Apesar dos ambientes metalíferos serem custosos para invasão e estabelecimento, os organismos que neles vivem desenvolveram adaptações evolutivas para lidar com os metais desses ambientes. Isso implica em acumular metais em seus tecidos sem danos ao crescimento (plantas) e reduzir o conteúdo de metais nos ninhos em relação ao solo adjacente (cupins).

TABELAS

Table 1: Elements concentrations (mg/Kg) in Parque Estadual do Itacolomi (PEIT) and Padre Viegas (PV) soils, in Minas Gerais state, Brazil. Ratio PEIT/ PV means how many times the element occurs in the soil of the PEIT in relation to the soil of PV.

Chemical elements (mg/Kg)	PEIT	PV	Ratio (PEIT/PV)
Al – alumínio	87042	15522	5.6
As – arsênio	<7,58	<7,58	
Ba – bário	10.32	2.47	4.2
Be – berílio	<0,137	<0,137	
Bi – bismuto	<4,9	<4,9	
Cd – cádmio	0.547	<0,491	1.1
Co – cobalto	12.88	2.51	5.1
Cr – cromo	306	82.97	3.7
Cu – cobre	99.65	11.77	8.5
Fe – ferro	187154	42505	4.4
Mn – manganês	753	92.16	8.2
Mo – molibdênio	<1,22	<1,22	
Ni – níquel	22.95	4.98	4.6
P – fósforo	529.82	57.82	9.2
Pb – chumbo	13.18	9.24	1.4
S – enxofre	434	226	1.9
Sc – escândio	16.49	9.68	1.7
Sr – estrôncio	<0,304	<0,304	
Th – tálio	31.59	10.51	3.0
Ti – titânio	378	1108	0.3
V – vanádio	104	102	1.0
Y – ítrio	0.94	<0,188	5.0
Zn – zinco	150	6.946	21.6
Zr – zircônio	75.44	5.05	15.0

Table 2 - Percentage and volume of soil used in each combination to compond the 7 levels and metal concentration (mg/Kg) in the soils of each level at which the plants (*Eremanthus erythropappus*) were cultivated.

Soil levels	% soil		Soil volume (L)		Metal concentrations in each soil level (mg/Kg)				
	PV	PEIT	PV	PEIT	Al	Cu	Fe	Mn	Zn
1	100	0	8.0	0.0	15522.00	11.77	42505.00	92.16	6.95
2	85	15	6.8	1.2	33402.00	33.73	78667.25	260.37	42.72
3	70	30	5.6	2.4	44130.00	46.92	100364.60	356.50	64.17
4	55	45	4.4	3.6	51282.00	55.71	114829.50	422.58	78.48
5	40	60	3.2	4.8	58434.00	64.47	129294.40	488.66	92.78
6	25	75	2	6	69162.00	77.68	146782.28	587.79	114.65
7	0	100	0.0	8.0	87042.00	99.65	187154.00	753.00	150.00

Table 3 - Initial and final height of Candeia, Candeias' growth rate, percentage of consumed leaf area, number of leaves attacked and metal concentrations in the Candeias' leaves.

Plant	Soil level	Initial height	Final height	Growth rate	Consumed leaf area (%)	Attacked leaves	Metal concentrations in Candeia's leaves (mg/kg)				
							Cu	Fe	Zn	Mn	Al
1	1	26	146	48	0.4	6	12.6	1079.45	115.85	823.75	302
2	1	38	114	108	0.0	5	7.7	817.7	110.65	653.35	211.15
3	1	34	90	75.5	0.0	1	8.2	704.7	122.3	1607.5	156.19
4	1	36.5	150	112	0.1	7	20.5	878.75	123.6	1670	217.49
5	1	34	142.5	77	4.8	8	20.75	1057.3	99.25	598.1	220.6
6	1	33	71	86	0.0	1	9.55	629.9	114	638.45	178.65
7	1	37.5	90.5	98.5	4.6	11	18.8	561.6	119	839.95	206.15
8	1	36.5	165.5	77.5	0.0	6	11.75	413.7	108.6	854.4	185.8
9	1	22.5	87	85	2.8	27	12.45	413.7	116.95	1080.5	196.2
10	1	33	112	120	0.5	16	12.8	320.95	97.7	717.15	137.1
11	2	35	158.5	62	0.0	7	8.55	513.65	112.65	1536.5	260.75
12	2	32.5	121	57.5	3.9	0	14.82	558.89	115.08	1183.59	469.6
13	2	34	125	88	0.0	4	14.35	426.55	112.2	1025.5	207.8
14	2	35.5	138	47.5	1.2	9	18.2	502.35	118.3	1501	273.45
15	2	9.5	148.5	114.5	2.8	5	15.2	476	113.1	817.9	250.2
16	2	30.5	153	73.5	0.1	8	15.55	475.75	120.75	1394	200.35
17	2	35.5	122	50	0.0	0	7.85	514.5	109.4	886.65	240.88
18	2	32.5	83	84.5	0.9	5	13.05	443.2	105.35	818.35	201.85
19	2	26.5	141	123.5	0.1	4	16.35	446.15	117.1	1691.5	158.4
20	2	31.5	104.5	18.5	1.5	3	11.05	365.15	69.25	728.65	128.65
21	3	7.5	113	64.5	0.7	7	12.65	531.85	112.9	1336	274.55
22	3	34	142	50	0.1	5	25.65	503.1	123.75	2344	269.4
23	3	31	110	111.5	0.2	4	17.1	344.7	108.5	1977	182.05
24	3	35	106.5	36	1.4	6	14.1	288.1	107.25	1471.5	223
25	3	27.5	216	113.5	2.4	10	25.95	375.75	115.05	2076	230.35
26	3	33	112	98	1.9	11	13.35	388.05	111.85	1772.5	197.15
27	3	23	142.5	81.5	5.2	9	11.85	307.35	109.25	1945.5	165.15
28	3	27.5	77	79	0.0	10	14.65	573.65	116.05	1276	208.95
29	3	34.5	90	55.5	0.0	3	12.85	382.15	87.9	805.3	161.85

Continua...

Plant	Soil level	Initial height	Final height	Growth rate	Consumed leaf area (%)	Attacked leaves	Metal concentrations in Candeia’s leaves (mg/kg)				
							Cu	**Fe**	**Zn**	**Mn**	**Al**
30	3	25	107.5	105	1.2	6	12.05	293.3	110.6	1300	125.4
31	4	8.5	124	82	0.0	4	9	452.95	101.7	1185.5	233.9
32	4	33	104	80	4.0	8	11.4	266.1	88.35	1315	151.05
33	4	34.5	72	103	1.4	10	18.15	435.5	102.6	2294	179.4
34	4	29	106.5	100.5	0.1	10	11.6	344.9	107.85	2251	160.55
35	4	37	129.5	71	2.3	13	12.95	410.45	116.15	1738.8	184.9
36	4	36	96.5	73	0.0	6	21	416.45	113.5	1586	307.3
37	4	26	96	70.5	0.1	3	23.55	414.35	121.55	2775	219.6
38	4	35	111	107	2.6	9	10.65	285.1	111.05	2561.5	178.75
39	4	34	184	131.5	0.7	5	12.85	285.95	117	1730.5	141.4
40	5	12	133	78	0.9	5	12.8	295.4	110.8	1533	157.15
41	5	30	122	86	0.0	2	9.15	334.05	103.15	1255.5	162.4
42	5	33	180	54	0.3	10	6.2	353.8	83.9	1533	197.55
43	5	31.5	109.5	78.5	0.4	12	11.5	338	107.6	1151	165.8
44	5	24.5	111	71.5	0.2	5	14.8	452.3	110.7	1069.5	229.7
45	5	33.5	85.5	125.5	0.5	11	11.85	288.35	110.65	1278	143.95
46	5	31.5	50	80.5	0.0	6	12.35	369.95	119.65	1645.5	178.25
47	5	30	64	67	0.1	12	19.45	339.65	118.5	1672	188.9
48	5	33.5	130	77.5	1.3	4	14.89	509.51	123.24	1895.69	257.1
49	5	32	109	74.5	1.2	31	14.95	380.55	114.1	1830.5	198.6
50	6	35.5	116	123	0.5	3	12.85	300.5	107.1	1208.5	178.1
51	6	41	159	143	1.5	18	16.6	452.2	112.15	1763.5	218.25
52	6	33.5	216	99.5	0.3	19	19.9	441.1	100.35	1783	179.75
53	6	31	154	62.5	0.2	4	9.15	341.9	86.4	1128	166.45
54	6	34.5	147	155.5	0.6	11	27.15	232.75	109.55	1913	161.95
55	6	35	74	181	3.2	39	19.75	283.8	112.45	1328	154.85

Continua...

Plant	Soil level	Initial height	Final height	Growth rate	Consumed leaf area (%)	Attacked leaves	Metal concentrations in Candeia's leaves (mg/kg)				
							Cu	Fe	Zn	Mn	Al
56	6	35	119	77	0.8	13	19.75	316.1	94.5	899.7	144.9
57	6	27	97	94	0.2	7	15.35	280.3	58.4	781.2	118.65
58	6	33	117	96	0.3	3	11.35	197.15	107.25	1118.5	151.15
59	7	33	84	109.5	0.4	23	11.8	224.5	108.55	1416.5	145.1
60	7	32	131	113	0.2	5	13.85	310.1	105.2	1356.5	145.15
61	7	36	137.5	86	0.5	13	17.13	349.05	116.6	1515	194
62	7	33	108	121	0.4	4	11.05	266.1	97.65	1168	162.85
63	7	38	90	87	0.3	15	13.2	323.3	121.05	2009	200.7
64	7	32.5	97	44.5	0.3	13	7.75	282.3	73.15	893.25	143.1
65	7	30	190	144	0.0	12	18.7	300	95.7	1632.5	217.15
66	7	27	129	153	0.4	19	13.95	234.75	99.35	1557	136.2
67	7	33	145	114	0.3	11	12.2	286.8	86.25	2214	165.25
68	7	39	174	99	0.1	5	10.45	305.55	78.25	1202.5	155.3

Table 4 - List of termitophiles found in the 16 nests of *Cornitermes cumulans* collected in Parque Estadual do Itaolomi (PEIT) and Padre Viegas (PV) in Minas Gerais, Brazil.

Order	Family	Specie	PEIT	PV
Acari	Not identified	Acari sp1	11	42
Acari	Not identified	Acari sp2	1	2
Acari	Not identified	Acari sp3		17
Acari	Not identified	Acari sp4		1
Acari	Not identified	Acari sp5		3
Acari	Not identified	Acari sp6	1	1
Acari	Not identified	Acari sp7		1
Araneae	Pholcidae	Pholcidae sp1		13
Araneae	Scytodidae	Scytodidae sp1	1	67
Araneae	Ctenidae	Ctenidae sp1		2
Araneae	Corinnidae	Corinnidae sp1		9
Araneae	Lycosidae	Lycosidae sp1		1
Araneae	Araneae	Araneae sp1		2
Araneae	Araneidae	Araneidae sp1		4
Araneae	Salticidae	Salticidae sp1		1
Araneae	Linyphiidae	Linyphiidae sp1		1
Araneae	Gnaphosidae	Gnaphosidae sp1		2
Araneae	Gnaphosidae	Gnaphosidae sp2		2
Araneae	Theridiidae	Theridiidae sp1		2
Araneae	Theridiidae	Theridiidae sp2		2
Araneae	Filistatidae	Filistatidae sp1		2
Araneae	Linyphiidae	Linyphiidae sp1		6
Araneae	Linyphiidae	Linyphiidae sp2		1
Araneae	Linyphiidae	Linyphiidae sp3		4
Araneae	Ctenidae	Ctenidae sp2		1
Araneae	Theraphosidae	Theraphosidae sp1		2
Araneae	Ctenidae	Ctenidae sp3		1
Araneae	Araneidae	Araneidae sp2	1	
Araneae	Gnaphosidae	Gnapnahosidae sp2	1	
Araneae	Salticidae	Salticidae sp2	1	
Araneae	Theraphosidae	Lasiodora	4	
Araneae	Araneidae	Araneidae sp3	1	
Araneae	Gnaphosidae	Gnaphosidae sp3	2	
Araneae	Sparassidae	Sparassidae sp1	1	
Araneae	Theridiidae	Theridiidae sp3	1	
Araneae	Gnaphosidae	Gnaphosidae sp 4	1	
Araneae	Gnaphosidae	Gnaphosidae sp5	1	
Araneae	Araneae	Mygalomorphae	1	
Araneae	Actinopodidae	Actinopididae	1	
Araneae	Gnaphosidae	Gnaphosidae sp6	1	
Araneae	Linyphiidae	Linyphiidae sp3	1	1
Araneae	Araneae	Araneae sp2	1	
Araneae	Linyphiidae	Linyphiidae sp4	1	
Araneae	Corinnidae	Corinnidae sp2	1	
Araneae	Linyphiidae	Linyphiidae sp5	26	
Araneae	Araneae	Araneae sp3	1	

Continua...

Order	Family	Specie	PEIT	PV
Blattodea	Blattodea	Blattodea sp 1	8	2
Coleoptera	Not identified	Coleoptera sp1	1	23
Coleoptera	Not identified	Coleoptera sp2		1
Coleoptera	Not identified	Coleoptera sp3	2	1
Coleoptera	Not identified	Coleoptera sp4		1
Coleoptera	Not identified	Coleoptera sp5	2	
Coleoptera	Not identified	Coleoptera sp6	5	
Coleoptera	Not identified	Coleoptera sp7	1	
Coleoptera	Not identified	Coleoptera sp8	1	
Coleoptera	Not identified	Coleoptera sp9	1	
Coleoptera	Elateridae (larvae)	Elateridae sp1	3	202
Coleoptera	Elateridae (larvae)	Elateridae sp2	8	
Coleoptera	Elateridae (larvae)	Elateridae sp3		1
Coleoptera	Elateridae (larvae)	Elateridae sp4		1
Coleoptera	Elateridae (larvae)	Elateridae sp5	1	
Coleoptera	Scarabaeidae	Scarabaeidae sp1	10	3
Coleoptera	Scarabaeidae	Scarabaeidae sp2	2	
Coleoptera	Scarabaeidae	Scarabaeidae sp3	16	
Coleoptera	Scarabaeidae	Scarabaeidae sp4	4	
Coleoptera	Staphylinidae	Staphylinidae sp1	5	8
Coleoptera	Staphylinidae	Staphylinidae sp2		1
Coleoptera	Staphylinidae	Staphylinidae sp3	58	35
Coleoptera	Staphylinidae	Staphylinidae sp4		13
Coleoptera	Staphylinidae	Staphylinidae sp5		15
Coleoptera	Staphylinidae	Staphylinidae sp6	4	5
Coleoptera	Staphylinidae	Staphylinidae sp7		1
Coleoptera	Staphylinidae	Staphylinidae sp8	18	
Coleoptera	Staphylinidae	Staphylinidae sp9	1	
Coleoptera	Staphylinidae	Staphylinidae sp10	3	
Coleoptera	Staphylinidae	Staphylinidae sp11	6	
Collembola	Not identified	Collembola sp1	8	3
Collembola	Not identified	Collembola sp2		1
Dermaptera	Not identified	Dermaptera sp	1	
Diptera	Phoridae	Phoridae sp1		855
Diptera	Phoridae	Phoridae sp2	1	
Diptera	Phoridae	Phoridae sp3		1
Diptera	Phoridae	Phoridae sp4	1	
Diptera	Phoridae	Phoridae sp5	1	
Diptera	Phoridae	Phoridae sp6		1
Diptera	Phoridae	Phoridae sp7		260
Diptera	Phoridae	Phoridae sp8		100
Diptera	Not identified	Diptera sp1		1
Diptera	Not identified	Diptera sp2		1
Diptera	Not identified	Diptera sp3	1	
Diptera	Not identified	Diptera sp4		2

Continua...

Order	Family	Specie	PEIT	PV
Homoptera	Not identified	Heteroptera sp1	71	145
Heteroptera	Not identified	Heteroptera sp2	1	
Heteroptera	Not identified	Heteroptera sp3		4
Heteroptera	Not identified	Heteroptera sp4		4
Heteroptera	Not identified	Heteroptera sp5	5	
Heteroptera	Not identified	Heteroptera sp6	6	
Homoptera	Not identified	Homoptera sp1		1
Homoptera	Not identified	Homoptera sp2		1
Isopoda	Not identified	Isopoda sp 1	5	1
Isopoda	Not identified	Isopoda sp 2	7	3
Lepdoptera	Geometridae	Geometridae sp		1
Lepdoptera	Arctidae	Arctidae sp		1
Lepdoptera	Not identified	Lepdoptera sp1	1	
Lepdoptera	Not identified	Lepdoptera sp2		3
Neuroptera	Berothidae	Berothidae sp		1
Annelida	Olygochaeta	Olygochaeta sp	1	
Chilopoda	Not identified	Chilopoda sp1		1
Chilopoda	Not identified	Chilopoda sp2	1	
Chilopoda	Not identified	Chilopoda sp3		2
Chilopoda	Not identified	Chilopoda sp4	1	
Orthoptera	Not identified	Orthoptera sp1		1
Orthoptera	Not identified	Orthoptera sp2		11
Orthoptera	Not identified	Orthoptera sp3		4
Orthoptera	Not identified	Orthoptera sp4	1	
Orthoptera	Not identified	Orthoptera sp5	1	
Pseudoscorpiones	Not identified	Pseudoescorpiones sp	9	1
Psocoptera	Not identified	Psocptera sp		1
Squamata	Amphisbaenidae	Anphisbaenidae sp		1
Thysanoptera	Not identified	Thysanoptera sp		1

Table 5 - List of Formicidae species found in the 16 nests of *Cornitermes cumulans* collected in Parque Estadual do Itacolomi (PEIT) and Padre Viegas (PV) in Minas Gerais, Brazil. Each number refers to sampled colony.

Family	Sub-family	Specie	PEIT	PV
Formicidae	Myrmicinae	*Atta* sp		2
Formicidae	Formicinae	*Brachymirmex sp*		2
Formicidae	Formicinae	*Camponotus* (Mirmotrix)	1	
Formicidae	Formicinae	*Camponotus crassus*	1	
Formicidae	Formicinae	*Camponotus renggeri*	1	
Formicidae	Formicinae	*Camponotus renggeri aff ruffipes*	1	1
Formicidae	Formicinae	*Camponotus ruffipes*	1	
Formicidae	Formicinae	*Camponotus* sp	2	1
Formicidae	Myrmicinae	*Cephalotes pusillus*	1	
Formicidae	Myrmicinae	*Crematogaster curvispinosa*		1
Formicidae	Dolichoderinae	*Linepithema humile*	1	
Formicidae	Dolichoderinae	*Linepithema sp*	1	
Formicidae	Ponerinae	*Neopenera marginata*		1
Formicidae	Myrmicinae	*Pheidole flavens*		1
Formicidae	Myrmicinae	*Pheidole sp*	1	1
Formicidae	Myrmicinae	*Pheidole subarmata*	1	
Formicidae	Dolichoderinae	*Tapinoma melanocephalum*		2
Formicidae	Myrmicinae	*Wasmania auropunctata*		2

Table 6 - List of inquiline species found in the 16 nests of *Cornitermes cumulans* collected in Parque Estadual do Itacolomi (PEIT) and Padre Viegas (PV) in Minas Gerais, Brazil. Each number refers to sampled colony.

Order	Family	Gender	PEIT	PV
Blattodea	Termitidae	*Agnathotermes*		2
Blattodea	Termitidae	*Armitermes*		1
Blattodea	Termitidae	*Atlantitermes*	1	
Blattodea	Termitidae	*Diversitermes*		2

Table 7 – Higher (H), volume (V), cohabitants abundance (Abd) and richness (Ric), metals concentration (Al, Cu, Fe, Mn and Zn) and mean time to workers death of each nest collect at PEIT (Parque Estadual do Itacolomi) and PV (Padre Viegas), Minas Gerais state, Brazil.

Nest	Area	H (cm)	V (L)	Abd	Ric	metals concentration (mg/Kg)					
						*Al^{3+}	Cu	Fe	Mn	Zn	mean time
PEIT263	PEIT	56	132.22	54	19	0	0.97	30.42	253.57	3.34	1356.9727
PEIT270	PEIT	41	97.07	10	9	0.56	1.18	63.26	510.35	5.6	860.0426
PEIT273	PEIT	45	41.9	63	9	0.28	1.41	86.01	67.94	2.11	993.5295
PEIT279	PEIT	42	69.85	39	8	0.38	1.18	44.39	106.27	3.63	886.7838
PEIT456	PEIT	23	17.24	17	5	0	2.06	73.87	600.31	8.02	415.3081
PEIT459	PEIT	35	33.51	34	11	0.38	1.1	44.17	193.52	3.23	589.0807
PEIT461	PEIT	57	189.55	42	3	0.28	1.23	92.74	374.97	8.58	439.9663
PEIT597	PEIT	70	207.74	110	41	0.94	1.78	61.95	156.07	3.44	729.2752
PV275	PV	67	202.71	197	20	0.28	1.25	70.89	37.72	3	1306.6673
PV276	PV	80	265.99	472	24	0.28	1.77	50.08	86.82	3.38	1337.66
PV376	PV	57	190.3	185	36	0.38	2.79	82.48	93.76	2.85	731.6719
PV377	PV	90	244.88	362	28	0.56	1.38	120.64	51.16	3.96	1370.9948
PV378	PV	56	210.43	204	26	0.38	2.81	72.08	91.16	2.88	1472.9601
PV380	PV	35	90.48	287	8	0.38	3.26	130.28	69.91	2.89	1264.1491
PV390	PV	30	51.37	111	15	0.28	2.19	60.81	48.34	3.71	1054.1666
PV391	PV	36	94.54	124	15	0.19	2.62	71	64.38	2.23	1482.8022

* O Al^{3+} was measured in cmol$_c$/dm^3 unit. The other metals (Cu, Fe, Mn and Zn), in mg/Kg unit.